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Begin the article with a short summary.

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Submit each table on a separate page, typed double spaced. Please use standard abbreviations; otherwise spell out.

References
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The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through November 30, 1976.

Combined Modality Treatment for Oat Cell Carcinoma of the Lung: A Randomized Trial [1,2,3]

Michael Alexander, Eli J. Glatstein, David S. Gordon, and John R. Daniels [4,5,*]

SUMMARY

Twenty-three patients with oat cell cancer of the lung were randomized to receive chemotherapy with POCC (procarbazine, 100 mg/m²/day orally, Days 1–14; vincristine, 2 mg iv, Days 1 and 8; cyclophosphamide, 600 mg/m² iv, Days 1 and 8; and CCNU, 60 mg/m² orally, Day 1) or COM (cyclophosphamide, 2000 mg/m² iv, Day 1; vincristine, 2 mg iv, Day 1; and methotrexate, 30 mg/m² iv, Day 15). After two to three cycles of chemotherapy, all patients were to receive radiotherapy to initially involved sites and then have chemotherapy continued. Patients treated with POCC had a median survival of 14 months vs 10 months for those treated with COM (P = 0.055). Eight of 15 first sites of relapse were intrathoracic and five of these eight had received radiotherapy to that site. Six central nervous system (CNS) failures were evenly divided between the two chemotherapy programs. Thus, the CNS penetration of procarbazine and CCNU in the POCC combination did not prevent CNS relapse. All future patients will receive prophylactic brain radiotherapy.

[Cancer Treat Rep 61:1–6, 1977]

In late 1972, Stanford University Hospital initiated a phase I–II trial to establish a combination chemotherapy regimen for oat cell carcinoma of the lung. The four-drug combination of procarbazine, vincristine (Oncovin), cyclophosphamide, and CCNU (POCC) utilized drugs known to produce regressions in oat cell carcinoma (1). Since procarbazine and CCNU enter the central nervous system (CNS) and have activity against primary brain tumors (2), it was hoped that the POCC combination would prevent CNS relapses. Eagan et al (3) later published encouraging results in the treatment of oat cell carcinoma with chemotherapy utilizing cyclophosphamide, vincristine (Oncovin), and methotrexate (COM), combined with radiotherapy. Subsequently, Stanford initiated a randomized clinical trial to compare our chemotherapy regimen with COM. It was planned that all patients would also receive involved field radiotherapy. The results of that study are described.

MATERIALS AND METHODS

Twenty-three patients were entered into the study. Criteria for admission were: (*a*) a biopsy or sputum cytology demonstrating oat cell type of small cell undifferentiated carcinoma of the lung; (*b*) disease previously untreated with radiotherapy or chemotherapy; (*c*) a Karnofsky status of ≥ 30% (4); (*d*) no history of other malignant disease over the preceding 5 years (excluding skin cancer); and (*e*) an informed consent.

Staging was based on the International Union Against Cancer system as modified by the Radiation Therapy Oncology Group (5). Initial staging studies planned for all patients included: chest roentgenogram, metastatic bone survey, bone scan, posterior

[1]Received July 6, 1976; revised Dec 27, 1976; accepted Jan 4, 1977.

[2]Presented at the 12th Annual Scientific Meeting of the American Society of Clinical Oncology, Toronto, Canada, May 1976.

[3]Supported by Public Health Service grants CA-05838, CA-08122, and CA-05289 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[4]Divisions of Oncology (Drs. Alexander and Daniels) and Radiation Therapy (Dr. Glatstein), Stanford University School of Medicine, Calif, and Clinical Immunology Laboratory (Dr. Gordon), Center for Disease Control, Atlanta, Ga.

[5]We thank Dr. Vincent T. DeVita for suggestions leading to the initiation of this study and Dr. John Fuery for generous referral of patients.

*_Reprint requests to:_ John R. Daniels, MD, S-025, Stanford Medical Center, Stanford, Calif 94305.

TABLE 1.—Chemotherapy schedules

Drug regimen	Route of administration	Dose/schedule
POCC*		
Procarbazine	Oral	100 mg/m²/Days 1–14
Vincristine	Iv	2.0 mg/Day 1 and 8
Cyclophosphamide	Iv	600 mg/m²/Day 1 and 8
CCNU	Oral	60 mg/m²/Day 1
COM†		
Cyclophosphamide	Iv	2000 mg/m²/Day 1
Vincristine	Iv	2.0 mg/Day 1
Methotrexate	Iv	30 mg/m²/Day 15

*Cycle repeated every 28 days.
†Cycle repeated every 21 days.

iliac crest bone marrow biopsy, liver scan, brain scan or computerized-axial brain tomographic study (CAT), serum chemistries, and complete blood cell and platelet counts.

Patients were randomized to receive POCC (12 patients) or COM (11 patients). Dose schedules and dose modification guidelines are shown in tables 1 and 2. The COM schedule was modified from that of Eagan et al (3) when it was found that methotrexate could be given on Day 15 rather than Day 21.

After two or three cycles of chemotherapy, radiotherapy was administered by a 4-MeV linear accelerator to sites of involvement demonstrated at initial staging regardless of response to chemotherapy. For disease limited to the mediastinal and hilar regions, a 5000–5500-rad mid-plane dose was given to a bilateral hilar, mediastinal, and supraclavicular port. The first 3000 rads were delivered by an opposed anterior-posterior technique with the remainder by isocentric technique using an anterior and a posterior oblique field. The dose rate was 1000 rads/week in four or five fractions. For patients with distant metastases, 3000 rads were given in 2 weeks to a hilar, mediastinal, and supraclavicular port by anterior-posterior opposed technique. Hepatic involvement was treated with 2500 rads in 3 weeks by anterior-posterior opposed technique. Pleural masses or effusions received 1500–2000 rads to the hemithorax over a 2–3-week period. For brain involvement, a 3000-rad mid-plane dose was delivered over 2 weeks via opposed, lateral fields.

After completion of radiotherapy, chemotherapy was to be continued until tumor progression was noted or until 2 years of disease-free survival had lapsed from initiation of treatment.

Responses were scored as complete (CR), partial (PR), and no response (NR). CR required disappearance of all measurable disease except that an initially abnormal bone scan was not required to return to normal. PR required a $\geq 50\%$ regression in the largest diameter of all measurable lesions with no appearance of new lesions. All lesser responses were classified as NR. No duration of response was required for classification as PR or CR.

Actuarial survivals were calculated according to the method of Berkson and Gage (6) and comparison of survival curves according to the method of Gehan (7).

RESULTS

Patient Characteristics

Comparison of patients receiving POCC and COM (table 3) shows the two groups to be similar in age, sex, disease stage, and Karnofsky status.

Survival

Actuarial survival curves are shown in figure 1. The median survival for the entire group is 11 months. The median survival for patients receiving POCC is 14 months compared to 10 months for those receiving COM. These curves are different at a $P =$

TABLE 2.—Dose modifications for cyclophosphamide, CCNU, and procarbazine*

Wbcs (mm³)	Platelets (mm³)	Calculated dose† (%)
≥ 3500	≥ 150,000	100
3000–3400	≥ 150,000	75
2500–2900	≥ 150,000	50
< 2500	≥ 150,000	0
≥ 3500	100,000–149,000	75
≥ 3500	75,000–99,000	50
≥ 3500	< 75,000	0
3000–3400	100,000–149,000	50
3000–3400	< 100,000	0

*Methotrexate and vincristine were given in full doses regardless of blood counts.
†If calculated dose for initiation of cycles is < 75%, delay initiation of cycle by 1 wk.

0.055 level. One patient is still alive in each group after 16+ and 19+ months.

Complete responders to chemotherapy had a median survival of 14 months vs 12.5 months for partial responders. The combined survival for CRs and PRs is longer (P = 0.03) than the 7-month median for NR patients.

Response to Chemotherapy (Tables 4 and 5)

Response rates were equivalent for POCC and COM with 15 (68%) of 22 evaluable patients in both groups having a CR or PR. One patient was not evaluable after removal of all known disease at thoracotomy. Another patient who completely responded to COM + radiotherapy relapsed during subsequent COM therapy and was the only patient crossed-over to POCC. He had a PR to POCC and is still alive 19+ months after diagnosis.

Response to Radiotherapy (Table 4)

Three patients were not evaluable, one because of removal of all known disease at thoracotomy. Two patients did not receive radiotherapy, one because of persistently positive bone marrow and the other because of fulminant disease progression. Although the proportion of patients with CR + PR was not improved after radiotherapy as compared to after initial chemotherapy (70% vs 68%, respectively), eight of 20 evaluable patients had further disease regression after radiotherapy while only three progressed. The number of patients with CR increased from five (23%) after chemotherapy to eight (40%) after radiotherapy. Of the three patients who had disease progression during radiotherapy, two had progression in unirradiated sites, one in bone with a previously normal bone scan, bone marrow biopsy, and alkaline phosphatase, and the other in the site of known chest disease while receiving hepatic and

TABLE 3.—Patient characteristics

	POCC	COM
No. of patients	12	11
Mean age in yrs (range)	58.5(46–74)	59.6(52–67)
No. of men	9	8
Mean Karnofsky status (range)	70(30–100)	66.3(30–100)
No. of patients (%) with disease stage—		
I	0	1(9)
II	1(8)	0
III	8(67)	6(55)
IV	3(25)	4(36)
Metastatic sites*		
Determined by		
Marrow biopsy	2/12	2/11
Bone scan	2/11	5/10
Liver scan	2/12	1/11
Brain scan, CAT	0/12	0/11
Pleura	1	1
Breast	1	0
Esophagus	0	1

*No. of patients with metastases/total No. of patients.

TABLE 4.—Treatment response

Treatment	No. of evaluable patients (%) with— CR	PR	NR	No. of patients not evaluable
Chemotherapy				
POCC	3(25)	5(42)	4(33)	0
COM	2(20)	5(50)	3(30)	1
Total	5(23)	10(45)	7(32)	1
Radiotherapy	8(40)	6(30)	6(30)	3

TABLE 5.—Initial site of relapse after response to treatment*

Site of relapse	No. of patients		
	POCC	COM†	Totals
Chest	2(2)	6(3)	8(5)
Bone	3(0)	1(0)	4(0)
CNS	2(0)	1(0)	3(0)
Liver	0	1(0)	1(0)

*Nos. in parentheses = No. of patients who had received prior radiotherapy to site of relapse.
†1 patient relapsed simultaneously in liver and chest.

brain irradiation. The third patient had progression of intrathoracic disease noted immediately after radiotherapy to that site.

Hepatic involvement was treated with radiotherapy in three patients, none of whom had a clinically evident hepatic relapse following radiotherapy. Post-mortem examination was performed in only one of the three patients and demonstrated hepatic involvement with tumor.

Sites of Relapse

Table 5 shows the first site of relapse for patients achieving a CR or PR. Eight patients were not evaluable because of inadequate response to treatment or because a specific site of relapse was not apparent. One patient had simultaneous relapse in the chest and liver. Of 15 patients in whom the initial site of relapse could be determined, three occurred during radiotherapy and 12 during chemotherapy. Of the 15 relapses, eight occurred in the thorax with five being in patients who had received irradiation to that site. Of these five, two had received 5000 rads, two 4000 rads, and one 3000 rads. The three patients who had not received chest radiotherapy included two with initial response and then progression with COM prior to radiotherapy, and one progression in the chest during hepatic and brain irradiation as noted previously.

Three patients suffered an initial relapse in the brain, two of whom had received POCC. Of three additional patients relapsing in the brain after first failing at another site, one had received POCC. Thus, the six CNS failures were evenly divided between the two chemotherapy arms.

Five patients had post-mortem examinations. These examinations invariably showed disseminated carcinoma involving multiple organ systems.

Toxicity

The incidence of leukopenia and thrombocytopenia with chemotherapy is shown in table 6. Leukopenia was common with both regimens. Thrombocytopenia was uncommon with COM but was the primary cause for treatment modification for POCC. Five of 11 patients experienced delays in initiation of a cycle of POCC; all were due to thrombocytopenia.

Infectious complications of chemotherapy were relatively uncommon. There were two episodes of bacteremia, one of which was associated with shock. No deaths were attributed to drug toxicity.

Duration of Treatment and Doses Administered

It was planned to continue treatment for 2 years. However, all patients either relapsed or decided to discontinue treatment prior to 2 years. Percentages of calculated doses given before and after radiotherapy are shown in table 7. Patients received a median of four cycles of POCC (range, two to seven) and four and one-half cycles of COM (range, one and one half to 11½). Since POCC requires a 4-week schedule and COM a 3-week schedule, this represents a nearly equal duration of chemotherapy for each group.

DISCUSSION

Among the histologic types of lung cancer, oat cell carcinoma represents a paradox. Although it is the most responsive to radiotherapy (8) and chemotherapy (1), it has the shortest survival (9,10). The dismal survival results with both surgery (10) and mediastinal radiotherapy (11) reflect the high incidence of disseminated disease at the time of diagnosis. Thorough use of staging techniques in these

TABLE 6.—Patients with leukopenia and thrombocytopenia*

Treatment	Wbcs (< 3500/mm³)†		Platelets (< 150,000/mm³)†	
	A cycle	B cycle	A cycle	B cycle
POCC	4/12	11/12	7/12	8/12
COM	5/11	8/11	3/11	1/11

*No. of patients with leukopenia and thrombocytopenia/total No. of evaluable patients.
†A = Day 1 of cycle; B = Day 8 of POCC and Day 15 of COM.

SURVIVAL PROBABILITY

TABLE 7.—Doses of chemotherapeutic agents given

	% of calculated dose (median)	
	Before radiotherapy	After radiotherapy
POCC		
Procarbazine	85	35
Vincristine	100	60
Cyclophosphamide	98	90
CCNU	100	90
COM		
Cyclophosphamide	100	86
Vincristine	100	100
Methotrexate	100	100

patients at presentation has demonstrated that metastatic disease can be detected in 75% by mediastinoscopy (12), in 40% by bone marrow biopsy (13), in 72% by bone marrow biopsy and bone scan (14), and in 44% by peritoneoscopy and liver biopsy (15).

Current therapeutic approaches to oat cell carcinoma have evolved through a series of clinical trials. A randomized trial in England showed that radiotherapy produced significantly better survival than surgery (11). The addition of cyclophosphamide chemotherapy to radiotherapy increases survival over that of radiotherapy alone (16). The recognition that response rates with combination chemotherapy appear superior to those with single-agent chemotherapy (17) resulted in the current approach which utilized both combination chemotherapy and radiotherapy (18,19).

The response rate and survival which we have achieved compare favorably to previously reported trials (7,12,18–36). Comparison of survival between our patients treated with POCC and COM suggests that POCC produces longer survival. The most recent report by Eagan et al (3) of the results with their most successful COM + radiotherapy regimen shows that all ten patients entered are alive with the longest followup at 11 months. Our median survival with POCC + radiotherapy is 14 months.

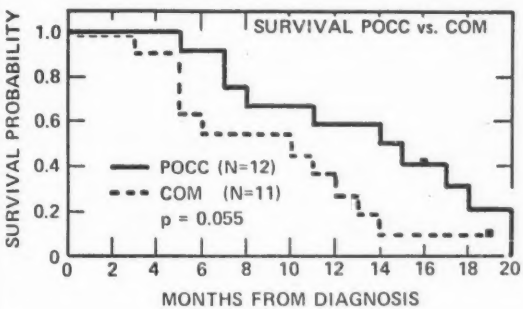

FIGURE 1.—Survival in patients treated with POCC + radiation vs COM + radiation.

It was hoped that POCC would prove effective in preventing relapses of oat cell carcinoma in the CNS. However, equal numbers of patients had CNS failure with POCC and COM. Because of this experience and the recognition by others of a high rate of CNS relapse (18,34), "prophylactic" brain radiotherapy was begun late in the POCC-COM protocol. Two patients receiving POCC and two receiving COM completed such treatment. None of these patients had a subsequent CNS relapse. An additional eight patients have received prophylactic brain radiotherapy on a subsequent ongoing protocol, and none has relapsed in the CNS during a short followup.

Our experience demonstrates that radiotherapy may improve the disease response seen with initial chemotherapy. However, as survival has been prolonged with combined chemotherapy-radiotherapy regimens, it is apparent that radiotherapy may not permanently control disease in treated sites. The fact that mediastinal oat cell carcinoma may respond dramatically to radiotherapy should not lead to the conclusion that the tumor mass has been sterilized. The uncommon recognition of recurrent mediastinal disease in irradiated patients in the past is undoubtedly a reflection of their short survival prior to the use of combination chemotherapy. Radiotherapists can expect to see significant rates of clinical recurrences in patients treated with radiotherapy if survival is prolonged by modern chemotherapeutic management. Despite thoracic radiotherapy, our most common site of initial relapse was the chest, perhaps simply reflecting the relative ease of detecting such relapses by chest roentgenogram. Although it is possible that these relapses resulted from re-seeding from other sites of persistent disease, these data challenge the presumed importance of radiotherapy in the treatment of advanced oat cell carcinoma.

Because of our interest in assessing the value of radiotherapy in such patients, randomization of POCC vs COM was discontinued after initial response rates were found to be equivalent with the two chemotherapy regimens. We have instead begun to study the usefulness of radiotherapy in patients with advanced oat cell carcinoma. All such patients receive POCC chemotherapy and prophylactic brain irradiation; half are randomized to receive additional radiotherapy to all known sites of involvement. It is hoped that this study will define the role of radiotherapy in advanced disease.

LIST OF COMPOUNDS

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

COM = cyclophosphamide, vincristine (Oncovin), and methotrexate

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

POCC = procarbazine, vincristine (Oncovin), cyclophosphamide, and CCNU

Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Cyclophosphamide L2 Protocol: A Combination Chemotherapeutic Regimen for Advanced Non-Hodgkin's Lymphoma [1,2,3]

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SUMMARY

A group of 49 patients with advanced non-Hodgkin's lymphoma were entered in a combination-chemotherapy protocol (cyclophosphamide L2). Of 14 patients with diffuse poorly differentiated lymphocytic lymphoma (DPDL), 64% responded with two partial remissions (PR) and seven complete remissions (CR). Both PRs are stable at 17+ months while six of the CR group are free of disease at 3+–23+ months. Fifty-three percent of 30 patients with diffuse histiocytic lymphoma (DHL) responded with 23% attaining CR status. Of the nine PR patients, six are stable at 11+–23+ months while six of the seven CR group are without disease at 9+–27+ months. The major toxic effect was bone marrow suppression with two deaths during periods of neutropenia; one of these deaths was definitely drug related. The encouraging results in the DPDL category have led to a continuation of this protocol for patients with this histologic type. In patients with DHL other approaches are being explored.

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Combination chemotherapy is responsible for increased response rates and prolonged survival in a number of neoplastic processes, notably childhood acute lymphocytic leukemia (ALL) (1,2) and Hodgkin's disease (3). Similar approaches are being taken in treating the non-Hodgkin's lymphomas with improving results. Wollner et al (4) developed a multiple-drug regimen which yielded substantially increased survival in children with non-Hodgkin's lymphoma. It was therefore decided to assess the efficacy of a similar protocol in adult patients on the Lymphoma Service at the Memorial Sloan-Kettering Cancer Center (MSKCC).

MATERIALS AND METHODS

Patients

All adult patients with advanced non-Hodgkin's lymphoma (stage III or IV by the Ann Arbor classification) of either the diffuse histiocytic (DHL) or diffuse poorly differentiated lymphocytic (DPDL) types were eligible for entry in the cyclophosphamide L2 protocol. The current report is restricted to those patients who were untreated prior to referral to the MSKCC or who had received only local radiotherapy or < 1 month of chemotherapy.

Pathologic material was reviewed by Drs. Phillip Lieberman and Daniel Filippa of the Department of Pathology and classified according to the system of Rappaport et al (5). Those patients with extensive or clinically aggressive disease in whom available tissue was inadequate to permit assignment to a Rappaport sub-group were placed in a separate category in assessing therapeutic response.

All patients were staged on the basis of history and physical examination, cbc count, urinalysis, biochemical screening profile (SMA-12), 5'-nucleotidase, serum protein electrophoresis, chest roentgeno-

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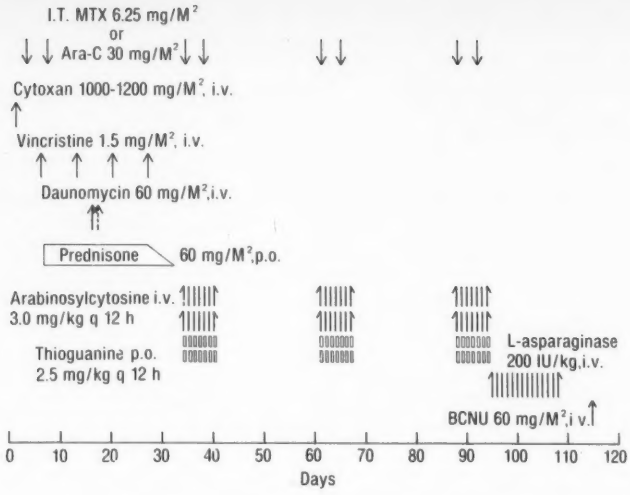

FIGURE 1.—Outline of cyclophosphamide L2 protocol.

gram, bone marrow aspiration and biopsy, lymphangiogram (unless contraindicated by extensive intrathoracic disease), and lumbar puncture. Laparotomy was not routinely performed for staging purposes. Cytologic or histologic confirmation was required before assigning a patient to the stage IV category.

Drug Administration and Dose Schedule

The cyclophosphamide L2 protocol is outlined in figure 1. It consists of a single bolus of cyclophosphamide (1200 mg/m² iv) on Day 1 followed on Day 3 by vincristine (1.5 mg/m² iv). The vincristine dosage is repeated three times with 7–10 days between each injection. Prednisone (20 mg/m² orally, tid) is begun 8 hours after the first vincristine injection and is continued for 21 days. It is then tapered over 3–4 days. Daunorubicin (60 mg/m² iv) is administered on 2 successive days between Days 12 and 23, the exact day determined by the degree of marrow suppression from the cyclophosphamide. Patients are routinely maintained on antacids and allopurinol during this phase of therapy. Prophylactic methotrexate (6.25 mg/m²) or cytosine arabinoside (30 mg/m²) is given intrathecally on the days indicated in figure 1 to all patients < 40 years of age and to all patients with bone marrow involvement.

Approximately 3–5 days after completion of the oral prednisone, cytosine arabinoside (3 mg/kg iv) and 6-thioguanine (2.5 mg/kg orally) are given every 12 hours to the point of marrow hypoplasia on bone marrow biopsy. A total of three courses of these two agents are given, with 2–3 weeks required between each cycle for full marrow recovery. Within 48 hours of the last dose of cytosine arabinoside and 6-thioguanine, L-asparaginase is given iv at 200 IU/kg/day for 7–14 days. Patients are monitored carefully for evidence of L-asparaginase toxicity. This initial stage of therapy is terminated with BCNU (60 mg/m²) 7 days after the last injection of L-asparaginase. For patients > 60 years of age the protocol is altered with the initial cyclophosphamide dose reduced to 1000 mg/m² and with only one daunorubicin injection. Areas of bulky disease or persistent tumor are treated with radiotherapy (500–3500 rads), usually between the first and second course of cytosine arabinoside and 6-thioguanine.

1.	6-Thioguanine	10 mg/kg orally × 4 days (Thurs–Sun)
	Vincristine	0.03–0.04 mg/kg iv × 1 (Mon)
	REST PERIOD TO ALLOW MARROW RECOVERY (10–14 days)	
2.	Hydroxyurea	60–80 mg/kg orally × 4 days (Thurs–Sun)
	Daunorubicin	1.0–1.5 mg/kg iv × 1 (Mon)
	REST PERIOD TO ALLOW MARROW RECOVERY (10–21 days)	
3.	Methotrexate	10 mg orally × 4 days (Thurs–Sun)
	BCNU	1.0–2.0 mg/kg iv × 1 (Mon)
	REST PERIOD TO ALLOW MARROW RECOVERY (10–21 days)	
4.	Hydroxyurea	60–80 mg/kg orally × 4 days (Thurs–Sun)
	Cyclophosphamide	20 mg/kg iv × 1 (Mon)
	REST PERIOD TO ALLOW MARROW RECOVERY (10–21 days)	

THEN RECYCLE

FIGURE 2.—Maintenance regimen for cyclophosphamide L2 protocol.

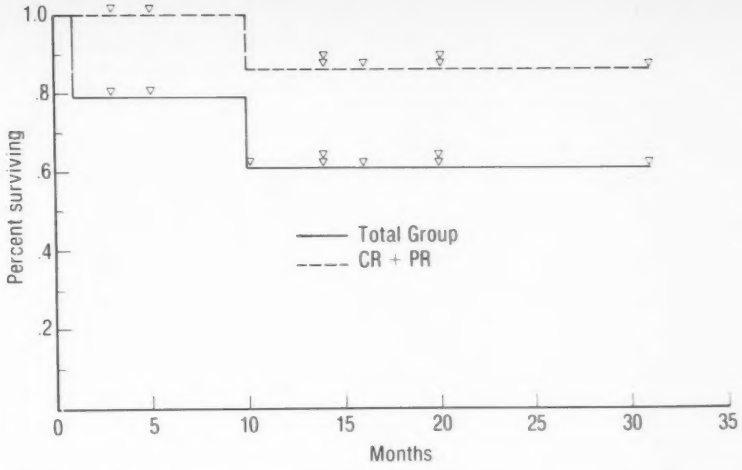

FIGURE 3.—Survival of patients with DPDL from initiation of chemotherapy. ▽ = time of last followup for a surviving patient.

Those patients who responded to this chemotherapy regimen then entered the maintenance phase (fig 2). This is a sequential program of seven cytotoxic drugs and is essentially the same as the maintenance arm of the L2 protocol for adult ALL (2,6). Maintenance therapy is continued for 3 years.

Assessment of Response

Those patients who died prior to receiving daunorubicin were considered to have had an inadequate trial of chemotherapy. A complete response (CR) was defined as the disappearance of all disease for a minimum of 1 month while a partial response (PR) was recorded when all measurable disease decreased by 50%. In some stage IV patients biopsies of involved viscera were repeated, but clinical evidence of regression was accepted in determining response status. For a patient to be considered a CR or PR, his performance status must have improved as well.

RESULTS

Study Patients

A total of 49 patients were entered in the cyclophosphamide L2 protocol: 14 had DPDL, 30 had DHL, and five had undifferentiated lymphoma. Clinical characteristics are outlined in tables 1–3. In the DPDL group there were four stage III and ten stage IV patients with five of the latter having bone marrow involvement. Of the patients with DHL, eight were stage III and 22 were stage IV with lymphoma shown in the marrow of 12. At the present time no patients have completed the maintenance phase of the protocol.

Therapeutic Response

Of the 14 patients with DPDL, 12 received an adequate trial of therapy with two attaining PR and seven attaining CR status. Two patients died soon after beginning the regimen, one on Day 3 of a pulmonary embolism (confirmed at autopsy) and the other on Day 40 of progressive central nervous system deterioration. The latter patient had been withdrawn from the protocol on Day 14 due to the severe neurologic disturbance. Extensive evaluation failed to establish a definite etiology (repeated cerebrospinal fluid examinations were negative for the presence of malignant cells) and an autopsy was not permitted.

The response rate (CR and PR) was 64% for the entire group of 14 patients and 75% for those 12 patients who had an adequate course of chemotherapy. Both patients with a PR are stable at 17+ months. There are six patients in the CR group still free of disease at 3+, 5+, 7+, 12+, 12+, and 23+ months. Survival following initiation of therapy is illustrated in figure 3 and remission duration in figure 4. These curves were obtained by the product-limit method (7).

TABLE 1.—Characteristics of patients with DHL

Patient No.	Age	Stage	Extranodal disease	Radiotherapy		Duration of response (mos)
				Before protocol	During protocol	
1	48	IV	Bone marrow	—	Whole brain, 3000 rads	0
2	61	IV	Pleura	—	Para-aortic nodes, 1350 rads	PR, 7
3	33	IV	Lung	—	Left lung, 3500 rads	CR, 19+*
4	42	III	—	—	Pelvic nodes, 2000 rads; para-aortic nodes, 1000 rads	PR, 12+*
5	52	IV	Skin	—		PR, 16+*
6	56	III	—	—	Abdominal port, 1500 rads	0
7	24	IV	Lung, pericardium, stomach	Mediastinum, 5000 rads	—	0
8	27	III	—	Abdominal port, 1550 rads	—	0
9	38	IV	Stomach, pancreas	Port and dose unknown	—	CR, 13+*
10	74	IV	Bone marrow	Right neck, 4000 rads	—	0
11	55	III	—	Right neck and axilla, 3000 rads	—	CR, 13+*
12	22	IV	Bone marrow	Right sacroiliac joint, 3000 rads	—	PR, 23+*
13	34	IV	Ovary	—	—	CR, 16+*
14	69	IV	Bone marrow	—	—	0
15	64	IV	Pleura	—	—	CR, 6
16	62	III	—	—	—	PR, 2
17	60	IV	Liver	—	—	0
18	44	IV	Skin, bone marrow	—	—	0
19	64	III	—	—	—	CR, 27+*
20	52	IV	Bone marrow	—	—	CR, 9+*
21	53	III	—	—	—	PR, 11+*
22	20	IV	Bone marrow	—	—	PR, 22+*
23	67	IV	Bone marrow	—	—	PR, 8
24	23	IV	Lung, liver, bowel	—	—	0
25	67	IV	Bone marrow	—	—	0
26	48	III	—	—	—	PR, 11+*
27	65	IV	Bone marrow, liver, adrenal	—	—	0†
28	73	IV	Bone marrow	—	—	0†
29	51	IV	Pleura, bone marrow	—	—	0
30	22	IV	Pleura	—	—	0

*Response is continuing.
†Inadequate trial of chemotherapy received.

TABLE 2.—Characteristics of patients with DPDL

Patient No.	Age	Stage	Extranodal disease	Radiotherapy		Duration of response (mos)
				Before protocol	During protocol	
1	65	IV	Bone marrow, colon, central nervous system	—	Whole brain, 600 rads	0
2	34	IV	Skin	—	Left forearm, 1600 rads; left thigh, 1600 rads; right inguinal nodes, 2000 rads	CR, 7+*
3	47	IV	Bone marrow, testicle	Right groin, dose unknown	—	CR, 4
4	26	III	—	Right inguinal nodes, 3500 rads; left inguinal nodes, 3000 rads; upper para-aortic nodes, 1425 rads; lower para-aortic nodes, 2550 rads; mediastinum, 3000 rads	—	PR, 17+*
5	16	IV	Cerebrospinal fluid	—	—	0
6	59	III	—	—	—	CR, 5+*
7	49	IV	Bone marrow	—	—	CR, 23+*
8	22	III	—	—	—	CR, 12+*
9	66	IV	Breast	—	—	0
10	65	IV	Pleura	—	—	PR, 17+*
11	28	IV	Bone marrow	—	—	CR, 12+*
12	17	IV	Bone marrow, pleura	—	—	CR, 3+*
13	66	IV	Testicle	—	—	0†
14	48	III	—	—	—	0†

*Response is continuing.
†Inadequate trial of chemotherapy received.

TABLE 3.—Characteristics of patients with undifferentiated lymphoma

Patient No.	Age	Stage	Extranodal disease	Radiotherapy		Duration of response (mos)
				Before protocol	During protocol	
1	19	III	—	—	Mantle, 4000 rads; pelvic port, 800 rads	PR, 23+*
2	20	IV	Bone marrow, central nervous system	—	Whole brain, 2400 rads	PR, 4
3	21	IV	Bone marrow	—	—	CR, 36+*
4	19	IV	Pleura	—	—	CR, 4+*
5	56	IV	Parotid	—	—	CR, 16+*

*Response is continuing.

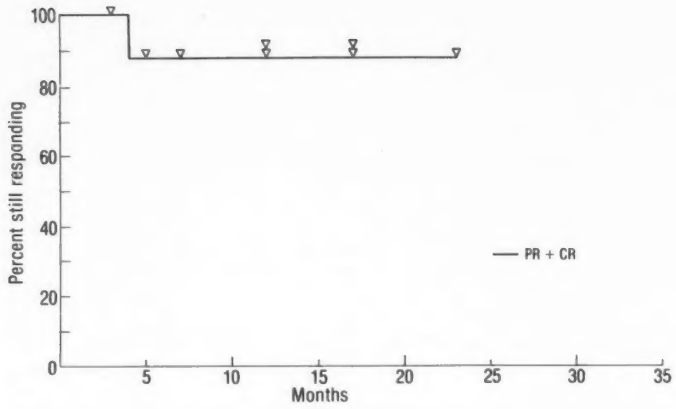

FIGURE 4.—Remission duration of patients with DPDL. ▽ = time of last followup for a responding patient.

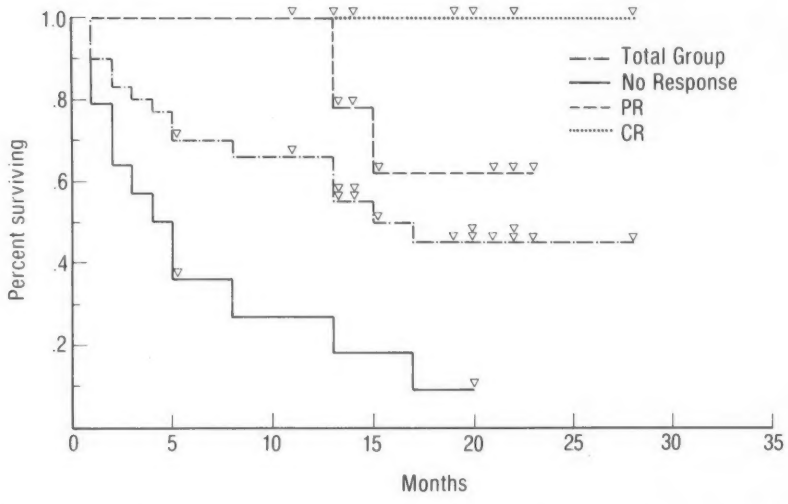

FIGURE 5.—Comparison of survival in subgroups of patients with DHL from entry in the protocol. ▽ = time of last followup for a surviving patient.

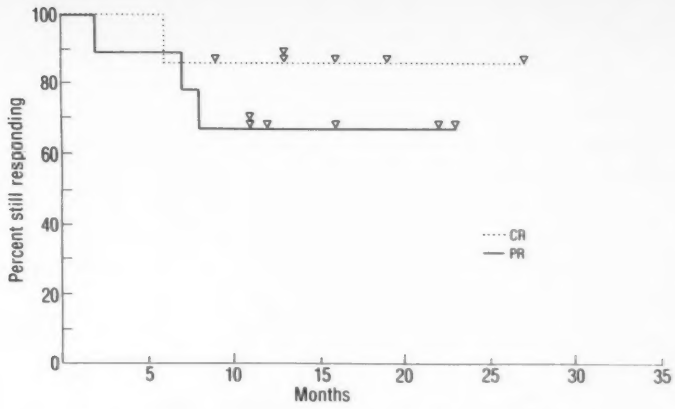

FIGURE 6.—Remission duration of patients with DHL. ▽ = time of last followup for a responding patient.

Twenty-eight of the 30 patients with DHL had an adequate course of chemotherapy with nine PRs and seven CRs. Two patients died before reaching the daunorubicin phase of the protocol. One patient died on Day 4 with extensive involvement of the liver, retroperitoneum, and bone marrow; the other died on Day 7. He, too, had extensive bone marrow infiltration and in addition at presentation had involvement of blood with a leukocyte count of 100,000/mm³ (95% immature lymphoid forms). On the day of his death his wbc count had fallen to 4500/mm³ with 95% blast forms.

For the group of 30 DHL patients the overall response rate is 53% while for the subset of 28 receiving an adequate therapeutic trial the response rate is 57% with 25% of the patients having a CR. In the CR group six patients are free of disease at 9+, 13+, 13+, 16+, 19+, and 27+ months while six PR patients continue unchanged at 11+, 11+, 12+, 16+, 22+, and 23+ months. Survival after the start of therapy is plotted in figure 5 and remission duration in figure 6. The median time of remission duration has not been reached in either the PR or CR groups.

A total of five patients were in the undifferentiated group. All were evaluable with two PRs and three CRs. Of the PR patients, one relapsed at 4 months while the other is stable at 23+ months. The CR patients are all still free of disease at 4+, 16+, and 36+ months.

Toxicity

As expected, there was substantial bone marrow suppression during the induction phase of the cyclophosphamide L2 protocol. Of the 49 patients, 27 had a neutrophil count of $< 1000/mm^3$ on at least 1 day. The nadir of the wbc count ranged from 100 to 3000/mm³ with a median value of 800/mm³. A total of 29 patients had one or more febrile episodes (temperature · 38.5°C for · 48 hours) with positive bacterial cultures in 12. There were two patient deaths from sepsis during a period of neutropenia. In one of these patients granulocytopenia was clearly drug related but in the other (*see* Therapeutic Response) it might have been due largely to extensive marrow involvement by DHL. Thrombocytopenia was frequently observed with 35 patients having a value of $< 100,000$ platelets/mm³ on at least 1 day and 27 of these having a count of $< 50,000/mm^3$. The median nadir platelet count was 30,000/mm³ and 13 patients received platelet transfusions. There were no significant bleeding episodes related to decreased platelet counts.

Bone marrow toxicity also occurred during the maintenance phase of therapy but to a lesser degree. Twenty-six of the 28 patients entering the maintenance protocol were followed in the Hematology Clinic at the MSKCC and detailed records are available. The nadir of the wbc count ranged from 400 to 8100/mm³ with a median of 1700/mm³. There were three febrile episodes necessitating hospital admission and in one case bacterial cultures were positive. Thrombocytopenia was documented with a value of $< 100,000$ platelets/mm³ in 17 patients and $< 50,000/mm^3$ in ten. The median nadir value was 84,000/mm³. Platelet transfusions were required by three patients. There were no deaths due to marrow suppression during the maintenance regimen.

High-dose steroids were probably responsible for complications in several patients. One patient had a

gastric perforation and another developed aseptic necrosis of the femoral and humoral heads.

Two patients developed abnormal liver function tests while receiving L-asparaginase. In both patients the HBAg was negative and the biochemical abnormalities resolved after discontinuing this medication.

Relapse

The sites of initial relapse following attainment of remission are listed in table 4. The patient with DHL who developed central nervous system disease had inadvertently not received the first two doses of intrathecal methotrexate.

TABLE 4.—Sites of initial relapse following remission

Histology	Site	No. of patients
DHL	Central nervous system	1
DHL	Breast	1
DHL	Nodes	1
DHL	Nodes, lung	1
DPDL	Marrow	1
Undifferentiated	Central nervous system, nodes	1

DISCUSSION

The decision to develop an aggressive multiple-drug protocol for advanced non-Hodgkin's lymphoma grew out of observations in childhood ALL in which induction of a remission state followed by maintenance therapy resulted in long-term survival and apparent cure in some patients (1). Similar concepts applied to childhood lymphoma by Wollner et al (4) also resulted in improved therapeutic results. Thus, an analagous regimen was designed for adult patients combining a number of drugs at full therapeutic dosages. It was realized that the protocol would have significant toxicity and would require prolonged hospitalization and close clinic followup in the induction phase. It was hoped that these negative features would be counterbalanced by increased remission rates and prolonged survival in comparison to more easily administered combinations.

The different classifications used in various institutions for staging and histologic categorization of the non-Hodgkin's lymphomas make direct comparisons between chemotherapy protocols difficult (8,9). However, the general adoption of the Rappaport system for pathologic analysis with modifications of the Ann Arbor staging classification has increased the uniformity of more recent series.

In patients with DPDL Stein et al (10) reported four PRs in five patients using vincristine and prednisone, while with cyclophosphamide, Oncovin (vincristine), procarbazine, and prednisone (COPP) there were three CRs among four patients. Berd et al (11) treated a single patient with a five-drug combination (cyclophosphamide, vincristine, methotrexate, citrovorum factor, and cytosine arabinoside) with a PR. A total of nine patients with DPDL were treated by Schein et al (12) with cyclophosphamide, vincristine, and prednisone (CVP) with two CRs and three PRs. The two CRs remained in remission at the time of publication at 9+ and 31+ months. The median response in the PR group was 2 months. Portlock and Rosenberg (13) obtained three CRs and one PR in four patients with stage IV DPDL treated with CVP and a response was obtained in one patient treated with CVP and bleomycin. An overall response rate of 64% was attained with the cyclophosphamide L2 regimen and a response rate of 75% was attained for the group of 12 patients with an adequate trial of therapy. While it is impossible to make definite conclusions based on the small numbers of patients available for analysis, it appears that the cyclophosphamide L2 protocol provides a response rate at least comparable to alternative combinations, although with greater toxicity. The ultimate value of this regimen will depend on the duration of remission. At the present time results have been sufficiently encouraging to continue entering patients with DPDL into this chemotherapeutic program.

A number of chemotherapeutic combinations have been used in advanced DHL. Stein et al (10) treated six patients with vincristine and prednisone with one PR and one CR. With COPP, one of six patients obtained PR status and another CR. The five-drug regimen described by Berd et al (11) was used in treating nine patients with one PR and six CRs. In a series of 27 patients, DeVita et al (14) reported an overall response rate of 70% with mechlorethamine, vincristine, prednisone, and procarbazine (MOPP) or C-MOPP (cyclophosphamide, vincristine, prednisone, and procarbazine) given for six monthly cycles. Of these patients, 41% had a CR with ten of 11 patients still free of disease on no maintenance therapy at the time of publication. These patients had a median response duration of 41 months. The Southeastern Cancer Study Group (15) used a cyclic combination of BCNU, cyclophosphamide, vincristine, and prednisone in treating 28 patients with DHL. The course of therapy was given monthly for six cycles with no maintenance. The overall response rate was 75% (14 CRs

and seven PRs) with eight patients remaining in CR with a median duration of 34.9 weeks. CVP was given to 13 patients with DHL, supplemented with bleomycin in three patients by Portlock and Rosenberg (13). They documented two CRs (15.4%) and five PRs (38.5%). Rodriguez et al (16) administered a combination of cyclophosphamide, adriamycin, vincristine, prednisone, and bleomycin to 35 adult patients with stage III or IV DHL. CVP was used as maintenance therapy once limiting doses of adriamycin and bleomycin had been given. The response rate was 92% and 66% were in CR at 1+–24+ months. The cyclophosphamide L2 protocol resulted in an overall response rate (CR + PR) of 53% in patients with DHL while in the subset with an adequate trial of therapy, the rate was 57% with 25% attaining a CR. Perhaps this response rate was lower than that in other series due to differences in referred patients. Forty percent of the patients with DHL treated at the MSKCC had marrow disease, a feature associated with a poor prognosis (17). Indeed, in only one of the present cases with bone marrow infiltration was a CR observed. However, it is possible that the number of CRs was actually higher than that recorded. In four patients, lytic bone lesions were documented radiologically with no alteration during therapy while other sites of disease fully resolved. These patients have been entered as PRs. Another three patients failed to attain CR status due to persistent abnormalities on lymphangiography of irradiated retroperitoneal nodes. These areas of disease showed marked initial improvement but did not return to normal. The difficulties in interpreting lymphangiograms of irradiated areas have been commented on by Dunnick et al (18). However, we do not feel that laparotomy is indicated in order to biopsy these nodes at present, especially since there would be no change in therapy.

Toxicity from the regimen was moderately severe and necessitated aggressive supportive management. There were two deaths due to sepsis during a period of neutropenia. One death was definitely drug induced while in the other, extensive marrow invasion by DHL probably contributed to the granulocytopenia. There were no major bleeding episodes during thrombocytopenic periods, probably due to the liberal use of prophylactic platelet transfusions. Prolonged periods of in-patient management were required, due to the poor initial clinical condition of many of these patients as well as the complicated nature of the protocol. In particular, re-admission was usually required for the courses of cytosine arabinoside and 6-thioguanine. At that point in therapy the majority of patients were fully ambulatory and were hospitalized only for medication.

The role of maintenance chemotherapy in the non-Hodgkin's lymphomas is not clear. DeVita et al (14) have reported unmaintained remissions in patients with DHL. They have proposed that patients still free of disease 24 months following termination of therapy may be considered cured. Durant et al (15) also followed a group of DHL patients with no maintenance treatment following CR. The median duration of unmaintained response was 34.9 weeks. Further followup will be required to determine the duration of such responses and for comparisons with series incorporating a maintenance phase.

Although both adults and children with non-Hodgkin's lymphoma received almost identical therapy, a comparison of the present results with those of Wollner et al (4) demonstrates a higher response rate in children. A superior response in children with ALL relative to adults treated with the same protocol has been documented by Gee et al (19) as well as by other investigators (20). Whether the difference is in the host response or in the nature of the tumor cannot be determined at present. Further analyses are being carried out in an attempt to define factors which may indicate which patients are likely to respond.

In view of the response rate which is no better than with other published regimens, as well as the toxicity and the prolonged in-patient management required, patients with DHL are no longer entered in this protocol. Other programs are being developed for this group. In DPDL the results are considered encouraging enough to warrant continuing the cyclophosphamide L2 protocol until a larger group of patients have been entered and longer followup is available.

LIST OF COMPOUNDS

Allopurinol: NSC-1390; CAS reg. No. 315–30–0; 1*H*-pyrazolo[3,4-*d*]-pyrimidin-4-ol

L-Asparaginase: NSC-109229; CAS reg. No. 9015–68–3

BCNU: NSC-409962; CAS reg. No. 154–93–8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

Cyclophosphamide: NSC-26271; CAS reg. No. 6055–19–2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Cytosine arabinoside: NSC-63878; CAS reg. No. 69–74–9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride

Daunorubicin: NSC-82151; CAS reg. No. 23541–50–6

Methotrexate: NSC-740; CAS reg. No. 59–05–2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

Prednisone: NSC-10023; CAS reg. No. 53–03–2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

6-Thioguanine: NSC-752; CAS reg. No. 1832–72–0; purine-6-thiol, 2-amino-

Vincristine: NSC-67574; CAS reg. No. 2068–78–2; leurocristine, sulfate (1:1)

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Enhancement of Antileukemic Effect in the Combination of 5-Fluorouracil and OK-432 [1,2,3]

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SUMMARY

OK-432, a streptococcal preparation with potent antitumor activity, has been evaluated for its efficacy in experimental and clinical trials. This preparation, however, was ineffective against mouse L1210 leukemia in all treatment schedules. The results of this study indicate that treatments with OK-432 and nucleic acid antimetabolites, such as 5-fluorouracil (5-FU), exert a synergistic effect against L1210 leukemia probably in conjunction with the immunologic defenses of the host. This therapeutic synergism was to some extent dependent on the dose level of 5-FU and was not produced against the 5-FU-resistant L1210 subline. In mice pre-treated with OK-432 prior to the leukemia implantation, there was no synergistic effect as a result of post-treatment with 5-FU. When BDF_1 mice had previously received X-ray irradiation or administration of corticosteroids, the synergism could not be expected either. Comparative combination therapy with BCG or group C streptococcus resulted in failure to produce therapeutic synergism in this system.

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Many reports have already been presented about the antitumor activity of a streptococcal preparation, OK-432, for which the antitumor action is due to either the direct cytotoxic effect on tumor cells in vitro and in vivo (1,2) or the potentiation of the reticuloendothelial system of the host, ie, host-mediated antitumor action (3,4). It is evident that OK-432 has brought about antitumor effects on various experimental tumors including spontaneously developed mammary carcinoma, chemically induced sarcoma, and drug-resistant tumors (1,2,5,6), and the clinical effect of this preparation has also been appraised(3,4,7–9). Although the mechanism of action of OK-432 is still obscure, the biologic character of this preparation seems to be different from that of simple or complicated polysaccharides from plants or basidiomycetes, and is also different from the biologic character of BCG, which is being studied for its systemic adjuvant properties.

Meanwhile, clinical usefulness has been prompted by the demonstration that OK-432 and other antitumor drugs acted synergistically against experimental tumors (3,4,10–15). Recently, an interesting result was obtained in our laboratory when the combination of OK-432 and inhibitors of nucleic acid biosynthesis were employed in the treatment of mouse L1210 leukemia (16).

This paper gives the defined data on therapeutic synergism in combined treatment with OK-432 and 5-fluorouracil (5-FU), and the characteristic properties of this preparation will be discussed.

MATERIALS AND METHODS

Experimental Animals

About 7-week-old female BDF_1 mice (C57BL/6 × DBA/2)F_1, weighing 19–22 g, were used in this study. The mice, which were obtained from the Shizuoka Laboratory Animal Agricultural Cooperative Asso-

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[3]5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8. Supplied by the Kyowa Hakko Kogyo Co, Ltd, Tokyo, Japan.

OK-432: NSC-402815; CAS reg. No. 39325-01-4; penicillin G, sodium salt. Supplied by the Chugai Pharmaceutical Co, Ltd, Tokyo, Japan.

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ciation, Hamamatsu, were randomized and housed in plastic cages in a constant temperature facility; they were maintained on a breeding diet CMF (Oriental Yeast Co, Ltd, Tokyo) and given water ad libitum.

Mouse L1210 leukemia was kindly donated by Dr. Hoshino of the Research Laboratory of Toyo Jozo Co, Ltd, Shizuoka, and has been maintained by ip passage at weekly intervals in BDF_1 mice in our laboratory.

Antitumor Drugs

Mitomycin C (Kyowa Hakko Kogyo Co, Ltd, Tokyo), nitrogen mustard *N*-oxide (Yoshitomi Pharmaceutical Co, Ltd, Osaka), cyclophosphamide (Shionogi and Co, Ltd, Osaka), 6-mercaptopurine (Kohjin Co, Ltd, Tokyo), methotrexate (Lederle Labs, Pearl River, NY), daunorubicin (Meiji Seika Co, Ltd, Tokyo), chromomycin A_3 (Takeda Chemical and Industrial Co, Ltd, Osaka), vinblastine (Eli Lilly, Shionogi and Co, Ltd, Osaka), podophyllic acid ethylhydrazide (Sankyo Co, Ltd, Tokyo), and BCG (lyophilized *Mycobacterium bovis* of Japan strain, Japan BCG Lab, Tokyo) were used. Cobalt protoporphyrin and cytosine arabinoside were generously supplied by the Midori Juji Co, Ltd, Osaka, and the Kohjin Co, Ltd, Tokyo, respectively.

In Vivo Antileukemic Experiments

L1210 ascites cells harvested from the mice bearing 6-day-old leukemia were diluted with physiologic saline to an appropriate concentration and then implanted ip (usually 10^5 cells/mouse) into BDF_1 mice. The treatment was started 24 hours after the leukemia cell implantation and continued for 7 successive days (Days 1–7). Each of the experiments was terminated on the 50th day after the implantation and survivors were killed and autopsied.

The antileukemic effect was evaluated by percent increase in lifespan (ILS) of the experimental animals over the control as follows:

$$\% \text{ ILS} = \frac{T - C}{C} \times 100$$

T = mean survival day of treated mice (excluding 50-day survivors)
C = mean survival day of control mice

RESULTS

Ineffectiveness on L1210 Leukemia of Treatment With OK-432 Alone

Sakurai et al (2) and Hoshino et al (5) have previously reported that OK-432 did not suppress the growth of L1210 leukemia and its drug-resistant

TABLE 1.—Influence of treatment schedule on OK-432 against L1210*

Treatment schedule	Route	Single dose (KE/kg)	Total dose (KE/kg)	Mean survival time† (days)	% ILS
Once, Day 1	Ip	2000	2000	Toxic	—
		1000	1000	8.2	2
		500	500	8.2	2
		250	250	7.8	−2
		125	125	8.0	0
q4h, Days 1,5	Ip	500	6000	8.2	2
		250	3000	8.0	0
		125	1500	8.2	2
		62.5	750	8.2	2
		31.2	375	8.2	2
qd, Days 1–6	Ip	1000	6000	7.8	−2
		500	3000	8.0	0
		250	1500	8.0	0
		125	750	8.0	0
	Sc	1000	6000	8.1	1
		500	3000	7.6	−5
	Iv	50	300	7.8	−2
		10	60	8.0	0
qd, Days −7 to −13	Ip	50	350	8.4	5
		12.5	87.5	8.2	2

*BDF_1 female mice received ip injections of 10^5 L1210 ascites cells on Day 0.
†The mean survival time of untreated control mice was 8.0 days.

sublines when 20–100 KE/kg[5] of OK-432 was injected into mice ip for 7 successive days or once every other day until death. However, more efficacious use of drugs in rodent tumor models is generally known to be dependent on treatment schedules, so treatment schedule-dependency tests with OK-432 against L1210 leukemia were carried out. Table 1 summarizes the results obtained in these experiments. As presented in this table, no antileukemic effect of OK-432 was observed in any of the treatment schedules examined so far. When OK-432 was given repeatedly prior to the leukemia cell implantation, this preparation was also found to be ineffective in contrast to the results obtained against Ehrlich carcinoma and ascites hepatoma 13 (2,4).

[5]Cell content in OK-432 was expressed as a Klinische Einheit (KE); 1 KE = 0.1 mg dried cells (approximately 10^9 cells) of streptococcal Su-strain.

From these results, it was concluded that pre- and post-treatment with OK-432 alone was entirely ineffective against mouse L1210 leukemia.

Combination Therapy With OK-432 and Other Antitumor Drugs

Although OK-432 was found to be ineffective against L1210 leukemia as described above, attention was focused on whether or not the improved therapeutic response for the combined use of OK-432 and other antitumor drugs is provided.

Antitumor drugs used in combination with OK-432 included some alkylating agents, antimetabolites, antibiotics, plant alkaloids, and others. In preliminary experiments, 50–75 KE/kg of OK-432 was injected ip 24 hours after the leukemia implantation followed by the administration of 1/2–1/3 optimal doses of the antitumor drugs 6 hours later. As

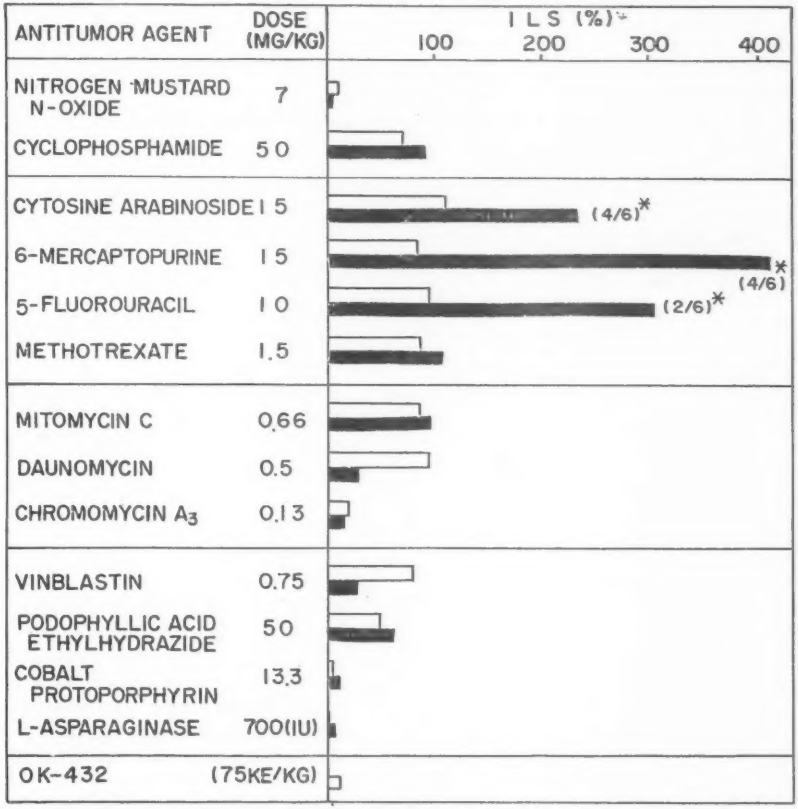

FIGURE 1.—Effect of combination therapy with OK-432 and other antitumor agents against mouse L1210 leukemia. Female BDF_1 mice which were implanted ip with 10^5 of L1210 cells received ip administration of antitumor agent alone (□) and in combination with OK-432 (■) for 7 successive days. * = 50-day survivors/no. of test mice.

shown in figure 1, when OK-432 and inhibitors of nucleic acid biosynthesis were employed in combination, the mean survival time of the treated mice was far greater than that for single-drug administration. The ILS was 233% with cytosine arabinoside plus OK-432, 413% with 6-mercaptopurine plus OK-432, and 300% with 5-FU plus OK-432, resulting in about half of the test mice surviving > 50 days after the leukemia implantation without tumor findings.

These results indicate that combinations of OK-432 and nucleic acid-antimetabolites are synergistic against L1210 leukemia, and that this therapeutic synergism may be explained by the concerted action of the drugs in immunologic and biochemical responses. Since the antitumor action of OK-432 seems to be partly due to the host-mediated action (2), the effect of pre-treatment with OK-432 was repeatedly examined in the following experiments: BDF_1 mice which received ip injections of OK-432 (25–50 KE/kg/day) for 10 successive days were implanted with L1210 cells in the 1st or 3rd week after the last injection followed by treatment with a daily dose of OK-432 (10 KE/kg) or 5-FU (10 mg/kg). The experiments indicated that the pre-treatment with OK-432 had very little or no effect on prolongation of lifetime of experimental animals even if additional treatments with 5-FU were given. Further investigations on the synergistic effect resulting from treatment with OK-432 and nucleic acid antimetabolites were carried out in defined protocols.

Proposal for the Combination Therapy With OK-432 and 5-FU

As described above, a remarkable antileukemic effect in the combination of OK-432 with an antimetabolite might be influenced by dose schedules, routes, or frequency of administration of both drugs, and by inoculum size of the L1210 cell. Experiments to provide a broad data base for conditions producing the therapeutic synergism were carried out. 5-FU, hereafter, was principally used in combination with OK-432, and was used in the form of mixed suspension each time.

Dose Schedules

A typical result obtained for dose schedules of both drugs is presented in table 2. As shown in this table, the dose response of OK-432 was not clear, but the dose of 5-FU was of decided importance and was necessary for optimal combination therapy from 6.2 to 12.5 mg/kg. This apparently indicates that the production of therapeutic synergism against L1210 leukemia was mainly dependent on the dose level of 5-FU but not so much on OK-432.

Routes and Frequency of Administration

The synergistic effect described hitherto was obtained in each case of ip injection of both drugs. If OK-432 acts as a nonspecific immunostimulator to the host, the route of administration of this agent

TABLE 2.—Dose schedule of combination therapy with OK-432 and 5-FU*

Treatment: Days 1–7 ip				
OK-432 (KE/kg)	5-FU (mg/kg)	Mean survival time† (days)	% ILS	50-day survivors/ No. of test mice
125	25	10.2	16	0/6
	12.5	19.8	125	0/6
	6.2	14.2	61	1/6
	3.1	11.8	34	0/6
25	25	12.5	42	0/6
	12.5	16.8	91	2/6
	6.2	20.5	133	2/6
	3.1	13.6	55	1/6
5	25	14.2	61	0/6
	12.5	16.8	91	1/6
	6.2	21.3	142	0/6
	3.1	13.5	53	0/6
None	25	15.0	70	0/6
	12.5	14.5	65	0/6
	6.2	14.2	61	0/6
	3.1	13.2	50	0/6

*BDF_1 female mice received ip injections of 10^5 L1210 ascites cells on Day 0.
†Excluding 50-day survivors. The mean survival time of untreated control mice was 8.8 days.

TABLE 3.—Effect of combination therapy with OK-432 and 5-FU by different routes of administration*

Schedule	Route: OK-432 (25 KE/kg/day)	Route: 5-FU (10 mg/kg/day)	Mean survival time† (days)	% ILS	50-day survivors/ No. of test mice
qd, Days 1–7	Ip	Ip	26.3	209	2/6
	Sc	Ip	15.8	86	0/6
	Iv	Iv	10.0	17	0/6
	—	Ip	14.0	65	0/6
	—	Iv	10.0	17	0/6
qd, Days 2–8	Ip	Ip	22.3	163	3/6
	Sc	Ip	16.0	88	0/6
	Iv	Iv	9.7	13	0/6
	—	Ip	14.3	69	0/6
	—	Iv	9.8	15	0/6
qd, Days 3–9	Ip	Ip	12.7	49	0/6
	Sc	Ip	12.8	50	0/6
	Iv	Iv	10.2	20	0/6
	—	Ip	13.3	59	0/6
	—	Iv	9.5	11	0/6

*BDF_1 female mice were given ip injections of 10^5 L1210 ascites cells on Day 0.
†Excluding 50-day survivors. The mean survival time of untreated control mice was 8.5 days.

TABLE 4.—Influence of sequential or intermittent administration of OK-432 in combination therapy with 5-FU

Experimental group	Treatment schedules: OK-432 (ip)	Treatment schedules: 5-FU (ip)	Mean survival time* (days)	% ILS	50-day survivors/No. of test mice
1	25 KE/kg; Days 1–9	10 mg/kg; Days 1–9	20.7	172	2/8
2	None	10 mg/kg; Days 1–9	13.3	75	0/6
3	100 KE/kg; Days 4,8,12	10 mg/kg; Days 1–11, except Days 4,8	16.0	111	3/8
4	None	10 mg/kg; Days 1–11, except Days 4,8	15.5	104	0/6
5	100 KE/kg; Days 1,5,9	10 mg/kg; Days 2–12, except Days 1,5,9	16.5	117	1/8
6	None	10 mg/kg; Days 2–12, except Days 1,5,9	12.2	61	0/6
7	25 KE/kg; Days 1–9	None	7.8	3	0/6
8	None	None	7.6	—	0/8

*Excluding 50-day survivors.

seems not so much to be in question, so that experiments regarding the available technique of administration site were carried out. The results shown in table 3 indicate that the synergism was produced only when both drugs were injected ip. Regardless of ip administration of 5-FU, sc administration of OK-432 failed to provide therapeutic synergism in the combination. Furthermore, the results of experiments about the time of the first treatment after the leukemia implantation are also presented in this table in connection with the administration route of the drugs. The therapeutic advantage was apparently provided when the combined treatment was started 24 or 48 hours after the implantation, but was not shown when leukemia invasion was advanced. Additional experiments, in which the frequency of administration of OK-432 in combination with 5-FU was examined, brought about evidence suggesting the possibility that antitumor activity of 5-FU may be concerted with immunologic capacity of OK-432. The treatment with 100 KE/kg of OK-432 once every 3 days for a total of three doses was found to be almost as effective as the treatment with 25 KE/kg of OK-432 for 9 successive days (table 4).

Inoculum Size of the L1210 Leukemia Cell

In previous experiments, the remarkable therapeutic advantage by combination therapy was obtained for the case in which limited inoculum size (10^5 cells) of the leukemia cell was adopted. Then, antileukemic experiments for a variety of inoculum

TABLE 5.—Correlation between inoculum size and antileukemic effect of combination therapy with OK-432 and 5-FU*

Inoculum size	Treatment†	Mean survival time (days)	% ILS
5×10^6	None	5.1	—
	5-FU	9.8	92
	OK-432 + 5-FU	10.0	96
1×10^6	None	6.1	—
	5-FU	11.6	90
	OK-432 + 5-FU	18.3	200
5×10^5	None	6.6	—
	5-FU	12.5	89
	OK-432 + 5-FU	20.2	206
1×10^5	None	7.8	—
	5-FU	13.3	70
	OK-432 + 5-FU	23.0	194

*BDF_1 female mice were given ip injections of L1210 ascites cells on Day 0.
†Days 1–7 ip: OK-432 (25 KE/kg/day); 5-FU (10 mg/kg/day).

TABLE 6.—Effect of OK-432 + 5-FU against L1210 in X-ray irradiated mice*

X-ray dose (rads)	Treatment†	Mean survival time (days)	% ILS
600	None	6.5	—
	OK-432	7.0	8
	5-FU	10.0	54
	OK-432 + 5-FU	10.6	63
300	None	7.4	—
	OK-432	8.0	8
	5-FU	15.4	108
	OK-432 + 5-FU	18.6	151
150	None	7.4	—
	OK-432	8.2	11
	5-FU	14.2	92
	OK-432 + 5-FU	20.8	181
0	None	8.0	—
	OK-432	7.2	−10
	5-FU	14.8	85
	OK-432 + 5-FU	23.4	192

*BDF_1 female mice were irradiated with X-rays generated at 180 KV, 15 mA, and filtered by 0.5-mm Al and 0.5-mm Cu (the dose rate was 64.5 rads/min at a focus target distance of 50 cm). 24 hrs later they were given ip inoculation of 10^5 L1210 ascites cells.
†Days 1–7 ip: OK-432 (25 KE/kg/day); 5-FU (10 mg/kg/day).

TABLE 7.—Influence of azathiopurine and dexamethasone upon antileukemic effect in combined use of OK-432 and 5-FU*

Pre-treatment	Post-treatment† Days 1–7 ip	Mean survival time‡ (days)	% ILS	50-day survivors/ No. of test mice
Azathiopurine (50 mg/kg/day), Days −8 to −2 orally	None	8.2	—	0/5
	OK-432	9.4	15	0/5
	5-FU	14.8	80	0/5
	OK-432 + 5-FU	15.3	87	1/5
None	None	8.4	—	0/5
	OK-432	9.4	12	0/5
	5-FU	13.8	64	0/5
	OK-432 + 5-FU	15.3	82	2/5
Dexamethasone (5 mg/kg/day), Days −8, −7 orally	None	8.0	—	0/5
	OK-432	8.3	4	0/5
	5-FU	13.1	64	0/5
	OK-432 + 5-FU	12.0	50	0/5
None	None	7.5	—	0/5
	OK-432	8.0	7	0/5
	5-FU	14.6	95	0/5
	OK-432 + 5-FU	20.4	172	1/5

*BDF_1 female mice received ip injections of 10^5 L1210 ascites cells on Day 0.
†OK-432, 25 KE/kg/day; 5-FU, 10 mg/kg/day.
‡Excluding 50-day survivors.

sizes were carried out to define the relationship between the number of leukemia cells implanted and the antileukemic effect. The results showed that therapeutic synergism by a combination of OK-432 with 5-FU was exerted when $\leq 10^6$ cells were implanted to mice, but was not when a larger number of the leukemia cells (5×10^6) were implanted (table 5).

Influence of Immunosuppressant on the Combination Therapy

The experiments, as presented in tables 6 and 7, were carried out in an attempt to demonstrate more fully the occurrence of the synergism between chemotherapy and immunity against L1210 leukemia. The therapeutic response observed in unloaded mice was remarkably abolished when mice had previously received whole-body X-ray irradiation (600 rads) or administration of a large dose of dexamethasone. Meanwhile, azathiopurine was found to slightly diminish the therapeutic effect brought about by the treatment with OK-432 and 5-FU. In another experiment, the influence of prednisolone and hydrocortisone acting on animals was less than that in X-ray irradiation or dexamethasone treatment.

Combination Therapy Against the 5-FU-Resistant L1210 Subline

In order to clarify that the therapeutic synergism resulting from the treatment with OK-432 and 5-FU is primarily due to the antimetabolic action of the latter, the antileukemic effect of this treatment was tested against the 5-FU-resistant L1210 subline. The results showed no effect in prolonging the lifespan of the experimental animals. This negative response was similarly observed in experimental animals either previously treated with OK-432 or untreated.

Combined Treatment With BCG and 5-FU

Considering the fact that potentiation of antileukemic action of 5-FU by OK-432 was not exhibited against the 5-FU-resistant subline, and also was not observed in the animals treated with immunosuppressants, the therapeutic synergism might be closely related to the antimetabolic effect of 5-FU, or the immunologic response of the host animal to OK-432 might also be potent enough to produce this dramatic effect. In this connection, it would be interesting to know whether or not BCG, which is well known to be a nonspecific immunostimulant, can produce therapeutic synergism against L1210

TABLE 8.—Antileukemic effect by combination of 5-FU and bacteria*

Treatment: Days 1–7 ip		Mean survival		50-day survivors/
Bacteria (25 mg/kg/day)	5-FU (10 mg/kg/day)	time† (days)	% ILS	No. of test mice
Group A streptococcus‡	+	19.3	176	3/6
	−	8.3	19	0/6
Group C streptococcus‡	+	14.6	108	0/6
	−	8.0	14	0/6
BCG	+	11.3	62	0/6
	−	7.8	12	0/6
OK-432 (25 KE/kg/day)	+	27.2	290	0/6
	−	8.2	17	0/6
None	+	14.0	100	0/6
	−	7.0	—	0/6

*BDF_1 female mice received ip injections of 10^5 L1210 ascites cells on Day 0.
†Excluding 50-day survivors.
‡Heated at 50°C for 30 mins.

leukemia by its combination with 5-FU. The results of comparative experiments with OK-432, BCG, and other bacteria are presented in table 8. Unexpectedly, no synergistic effect was obtained by the combination of BCG and 5-FU. On the contrary, when group A streptococcus, which is an active component of OK-432, was used in the combination, a remarkable synergism was clearly demonstrated. Such synergism was, however, not observed when 5-FU was combined with group C streptococcus, which is different from group A streptococcus in biologic properties. Furthermore, pretreatment with BCG followed by post-treatment with 5-FU, alone and in combination, was not so effective that demonstrable synergism was obtained by the combination of OK-432 and 5-FU.

Combination Therapy of Subcellular Fractions of Group A Streptococcus With 5-FU

If an active component in the OK-432 preparation for the production of therapeutic synergism is the streptococcal cell itself, it should be investigated using planned protocols for the intracellular products. In preliminary experiments, the crude materials of streptococcal cell fractions, ie, cell-wall fraction (trypsinized) and cytoplasmic-soluble fraction (supernatant at 105,000 × g centrifugation), which were prepared from live group A streptococcus (Su-strain) by a Ribi Cell Fractionator, were used in the experiments of combination therapy.

BDF_1 mice implanted ip with L1210 cells received ip administration of the streptococcal cell fractions alone or in combination with 5-FU for 7 successive days. The results presented in table 9 show that the treatment of cell-wall or cytoplasmic-soluble fraction alone was entirely ineffective against L1210 leukemia as well as treatment with OK-432 alone, and that the combination treatments of each cell fraction and 5-FU were found to be ineffective in the inhibition indicating therapeutic synergism. Other experiments carried out in parallel, using mice given 100 mg/kg ip of each cell fraction two times per week for 3 weeks, gave similar negative results.

DISCUSSION

Data have been previously presented to show that when OK-432 in aqueous suspension was treated at 90°C for 30 minutes, the antitumor activity of this preparation remarkably decreased (1), while the potency of the heat-treated preparation in potentiating the reticuloendothelial system in the animal was almost identical with that of the untreated one.[6] Sakurai et al (2) reported that the growth of Yoshida sarcoma and ascites hepatoma cells was inhibited by OK-432 in vitro, and the degree of inhibition was to some extent dependent on the dose level of OK-432. Niki and Takagaki (17) also reported that degenerative changes in intracellular organelles of Yoshida sarcoma cells came in contact with OK-432 in vitro. Moreover, Ono et al (18) and later Koshimura and Ryoyama (unpublished results) demonstrated that OK-432 inhibited the macromolecular syntheses, especially nucleic acid syntheses, of tumor cells in culture. These observations clearly indicate the effect of OK-432 acting directly on tumor cells.

[6]Takagaki Y. Personal communication.

TABLE 9.—Antileukemic effect of combination therapy with 5-FU and streptococcal cell fractions*

Treatment: Days 1–7 ip		Mean survival		
Agent	Single dose (mg/kg)	time† (days)	% ILS	50-day survivors/ No. of test mice
CW‡	25	8.8	14	0/6
SP§	25	9.2	19	0/6
5-FU	10	15.8	105	0/6
CW + 5-FU	25 + 10	13.8	79	0/6
SP + 5-FU	25 + 10	14.8	92	0/6
OK-432 + 5-FU	25¶ + 10	16.8	118	2/6

*BDF_1 female mice received ip injections of 10^5 L1210 ascites cells on Day 0.
†Excluding 50-day survivors. The mean survival time of untreated control mice was 7.7 days.
‡Cell-wall fractions of group A streptococcus.
§Cytoplasmic-soluble fractions of group A streptococcus.
¶KE/kg.

On the other hand, it was frequently observed that the animals pre-treated with OK-432 prior to tumor implantation rejected the takes of some transplantable tumors (2,4). Matsuno et al (19) reported that the antitumor effect of OK-432 was scarcely demonstrated in lethally irradiated or in antilymphocyte serum-treated animals.

From these results, the possibility was considered that the antitumor action of OK-432 could be in part dominated by the immune responses of the host. In spite of the accumulated data concerned with the antitumor activity of OK-432, the role of this preparation in cancer treatment is not yet clear. The planned protocols in this study were therefore arranged to more fully clarify the biologic properties of OK-432 as a unique antitumor agent. The correlation between tumor and host may appear to be an important factor in the investigation of immunotherapy. The mouse L1210 leukemia and the BDF_1 mouse $(C57BL/6 \times DBA/2)F_1$ were selected for use in the present study because the tumor is semisyngeneic to the host and suggestive evidence for a predictive value in the clinic may be obtained. The data obtained in this study seem to indicate that treatments with OK-432 and nucleic acid antimetabolites exert therapeutic synergism against L1210 leukemia in connection with the immunologic responses of the host.

The combination therapy with OK-432 and other antitumor drugs has been carried out experimentally and clinically, and the effectiveness of this combination therapy has been reported by many researchers (3,4,10–15). These reports have presented not only the usefulness of combination therapy but also the improvement of side effects caused by the application of chemotherapy in cancer patients (protection against decrease of leukocyte and blood platelet counts and potentiation of reticuloendothelial system, etc).

The synergism in the combination therapy of OK-432 with 5-FU was clearly demonstrable when OK-432 was successively or intermittently given during the treatment with 5-FU after the leukemia implantation. This synergistic effect seems to be due primarily to the action of 5-FU, because the synergism produced by the treatment with both drugs was dependent on the dose level of 5-FU and was not proved against the 5-FU-resistant L1210 subline. Such a synergistic effect was, however, depressed in mice receiving either administration of an immunosuppressant, such as dexamethasone or hydrocortisone, or sublethal X-ray irradiation. These results, considered together with those obtained in the previous experiments (1,19), suggest that the nonspecific immunopotentiating capacity of OK-432 may greatly contribute to the production of therapeutic synergism by the combination therapy.

Meanwhile, BCG, which is well known as a nonspecific immunostimulant, was found to be ineffective in the present experimental system, as well as in the experiments with group C streptococcus. However, there is some indication that combined use of an effective antitumor drug and BCG against experimental tumors is effective (20–22). Mathé et al (23) reported that only the "French fresh" Pasteur Institute preparation of BCG gave a significant result when ten preparations of BCG were submitted to the five tests of their systemic adjuvant screening battery; however, Sher et al (24) presented data to show that the difference in the BCG strain may not be of paramount importance in their experimental trials in which three strains (Pasteur, Japan, and Brazil) of BCG of varying virulence were tested for efficacy in chemoimmunostimulation of Moloney murine lymphoid leukemia. Further, it is likely that the technique of administration regarding site, dosage, frequency, and especially duration

in immunochemotherapy is important to produce the beneficial effect from cancer treatment.

In this regard, we cannot ignore the possibility that the differences in bacterial strain or in experimental system employed may dominate the results of this kind of experiment. Although there are many complicated problems in the field of immunotherapy, the evaluation of microbial products used in tumor immunotherapy should be carried out using fully planned protocols with various animal tumors.

Apart from the point described above, it has already been reported that the cell-wall fraction from group A streptococcus possesses immunostimulant properties and that cytoplasmic components from the streptococcus completely suppress the human lymphocyte transformation induced with phytohemagglutinin in vitro and inhibit the activity of immunocompetent cells to form antibodies against sheep erythrocytes in mice (25,26).

The results of additional experiments carried out with subcellular components of live group A streptococcus revealed that the combined treatment with 5-FU and cell-wall or intracellular-soluble fraction against L1210 leukemia resulted in a failure to produce the marked prolongation of mean survival days in experimental animals.

Although the mechanism of antileukemic synergism with a combination of an active and an inactive agent is very complicated, it is interesting that a possible therapeutic synergism against mouse L1210 leukemia was brought about by the combination of OK-432 and a nucleic acid antimetabolite. However, it is questionable that the degree of increased survival time is influenced by some factors, such as age, colony, or strain of the host animal. In this respect, further studies on the biologic and immunologic aspects of the therapeutic effect by combinations involving OK-432 on antitumor activity of subcellular fractions from live hemolytic streptococcus are currently under way.

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Pulmonary Metastases of the Lewis Lung Tumor—Cell Kinetics and Response to Cyclophosphamide at Different Sizes [1,2,3]

Richard P. Hill[4,5,*] and Judith A. Stanley [4,6]

SUMMARY

The response of lung metastases of the Lewis lung tumor to a single dose of cyclophosphamide (300 mg/kg) has been studied as a function of the size of the metastases. Both artificially induced and naturally arising (true) metastases were studied in the absence of a primary tumor. It was found that the artificial metastases exhibited a change in cure probability from 90% to 37% over a size range of a factor of ten. The true metastases had a larger spread in size for a similar change in cure probability but there were large uncertainties in the data and consequently the results are not inconsistent with those for the artificial metastases. Studies of the cell kinetics of the metastases at various sizes in the critical range for curability failed to demonstrate any marked differences. It was concluded that the results of drug treatment could be explained entirely on the basis of an increase in cell number in the larger metastases.

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The Lewis lung tumor growing either sc or im in its isogeneic host (C57BL mouse) metastasizes readily to the lung. Recent studies (1,2) on the curability of the tumor, using either combined surgery and chemotherapy or chemotherapy alone, have indicated that as the metastatic disease is allowed to become more advanced, treatment with drugs such as cyclophosphamide (CY) or 1-(2-chloroethyl)-3-(4-*trans*-methylcyclohexyl-1-nitrosourea) becomes less effective. The experiments described in this paper were designed to supplement these observations by determining the size range of pulmonary metastases which could or could not be cured by a single large dose of CY and to investigate whether the cell kinetic parameters of metastases in this size range could give any indication as to why the size range was critical. The experiments were carried out both with artificial metastases produced by injecting tumor cells iv into mice and true metastases arising naturally from tumors implanted im into the right hind leg of mice.

MATERIALS AND METHODS

Tumors and Mice

The Lewis lung tumor used in these experiments was obtained from Dr. K. Hellmann of the Imperial Cancer Research Fund (London, England) in December of 1971. It has since been maintained in C57BL mice of the Institute of Cancer Research colony. Its histologic and macroscopic characteristics are consistent with those reported by Sugiura and Stock (3). Female C57BL mice (8–12 weeks of age weighing 18–21 g) have been used throughout the experiments. The tumor has been serially passaged by injecting approximately 10^6 cells sc into the flanks of mice using the procedure described previously (4). None of these implants have failed to take but experi-

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[3]Cyclophosphamide: NSC-26271; CAS reg. No. 6055–19–2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate. Supplied by W. B. Pharmaceuticals Ltd, London.

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ments in which mice were injected with radiation-killed cells before being challenged with live cells suggested that a low level of antigenicity may exist for the tumor in this subline of C57BL mice (5). Such antigenicity has also been observed by others (6) with different sublines of both mice and tumor.

Production of Metastases

A single-cell suspension was prepared from sc tumors by mincing the tumor tissue and then trypsinizing it (4). To produce artificial lung metastases, 10^6 dye-excluding tumor cells or 1–4 × 10^5 tumor cells plus plastic microspheres were injected iv into recipient mice. The microspheres increased the number of tumor nodules formed in the lungs for a given number of cells injected (4). No difference was observed in the results obtained with artificial metastases generated in these two ways. True metastases were obtained by injecting 10^6 tumor cells im into the right calf muscle of recipient mice and then ablating the primary tumor with radiation at some time later—usually 7 days.

Radiation Treatment

The tumor-bearing legs were irradiated with ^{60}Co gamma rays at a dose rate of 250 rads/minute using a 13.5-cm thick lead shield to protect the body of the mouse (7). A dose of 6000 rads was given because previous work (7) had indicated that a dose of 5500 rads gave 100% cures of Lewis lung tumors 100 mm^3 in size. A similar set-up was used in some experiments to irradiate the tails of mice which had received iv injections of tumor cells. In this case, 3000–3500 rads were given 3 or 4 days after the injection of the tumor cells.

Drug Treatment

Cyclophosphamide was dissolved and diluted in phosphate-buffered saline (Dulbecco's Solution A) and injected ip. Each mouse received 6 mg in a volume of 0.5 ml, which is equivalent to a dose of approximately 300 mg/kg in these mice. This dose resulted in approximately 5% of the animals dying due to toxicity.

Labeling Studies

Mice bearing pulmonary tumors were injected with 20 microcuries of tritiated thymidine (Radiochemical Centre, Amersham, England; specific activity 10 curies/mM) ip and then killed at appropriate intervals. The lungs were quickly removed, washed with water to remove the blood, and fixed in 10% formalin in 0.9% NaCl solution. Sections 4-μm thick were cut and the slides were dipped in Ilford liquid emulsion and exposed for 3 weeks before being developed and stained with hematoxylin and eosin. In some batches of slides, sections from unlabeled tumors were included to allow an estimate of the background labeling of the slides. The criterion for labeling was set at 4 grains/nucleus. For the labeled mitoses studies, at least 100 mitoses were examined at each time point, except for the smaller nodules when this was not always possible, even though a number of different level cuts were examined for each nodule. For the larger nodules, complete sections were examined in order to obtain a representative count, and in cases where the lung sections examined contained more than one nodule, the nodules were studied independently. For labeling indices, sections from animals injected 1 or 2 hours before killing were used and at least 1000 cells in each of four nodules were examined for each time point.

Analysis of Drug Treatment Results

After drug treatment, mice were observed for 60 days and any mouse dying during this time was examined for metastases. At the end of the 60 days surviving mice were killed and similarly examined. No metastases were found in these surviving mice. Survival to 60 days was thus used as one criterion for assessing the response to drug treatment. Since this criterion naturally includes any deaths due to the toxic effects of the drug, a second criterion was also used which was the fraction of tumor recurrences after treatment. A number of mice did die early after the drug treatment, presumably as a result of CY toxicity. If the death occurred 14 days after treatment, the state of their metastases could not be assessed, and, consequently, they were rejected from the recurrence analysis but not from the survival analysis. Occasionally, in the early experiments mice with artificial metastases were found to develop tumors in the tail at the injection site. This was probably due to a trapping of cells in the tail of the mice as has been observed in rats by Van den Brenk (8). In the later experiments this problem was eliminated by irradiating the tail as described earlier. The mice which developed tail tumors were rejected from both the analyses.

The results were derived from a number of independent experiments, and in each experiment, 15–20 mice were not treated with drug but were kept as controls and used to assess the fraction of mice with true metastases or the number of tumor nodules per mouse for the artificial metastases. In most cases the number of tumor nodules was 15 per mouse.

For the experiments with true metastases, not every mouse in the group would be expected to have pulmonary metastases and hence, to determine the drug response, the fraction of control mice with lung metastases was used to determine the number of drug-treated mice expected to have tumors at the time of treatment. This expected number was then used as the number of mice at risk for the calculations of percentage survivors and tumor regrowth. Of course this procedure adds an additional source of error which is reflected in the size of the errors shown in figure 1(B).

RESULTS

Production of True Metastases

The advantage of using artificial metastases generated by injecting cells iv is that the tumor nodules are seeded at the same time and are fairly uniform in size. In order to study whether it was possible to simulate this uniformity of seeding with true metastases, Lewis lung tumor cells were transplanted into the right hind legs of a large number of recipient mice. At various times after this injection, the primary tumor was ablated by radiation. After irradiation the mice were split into two groups for each time interval; one group was killed 20 days after irradiation and the lungs were examined for macroscopic tumor nodules and the second group was kept for 60 days and any mice that died during this time were examined for macroscopic metastases. No mice which survived this length of time were found to have metastases when killed and investigated. These two groups were found to have the same proportion of mice with lung metastases at each of the times investigated. Consequently, in subsequent experiments the proportion of mice developing metastases was assessed by killing the mice 16–25 days after irradiation and examining their lungs macroscopically. The shorter time was found to be satisfactory in this regard from studies on artificial metastases (4).

The result of these experiments is shown in figure 2 where the percentage of mice developing lung metastases is plotted against the time of the irradiation after the im injection of tumor cells. No metastases were observed if the tumor-bearing legs were irradiated < 5 days after the injection. For irradiation times of > 5 days the percentage of mice developing metastases increased until, by 12 days after the injection, all the mice had developed metastases.

It can be seen from figure 2 that if the tumor-bearing legs were irradiated on Day 7 after the inoculation of tumor cells, then there would be a fairly narrow seeding period of 2–3 days and some 40% of the mice would develop pulmonary metastases. This was the procedure adopted for all the subsequent experiments with true metastases.

Growth of the Metastases

To determine the growth rate of the metastases, mice were killed at various times after either the iv or im injection of tumor cells and two perpendicular diameters of the pulmonary tumor nodules were measured in situ using a dissecting microscope (magnification, × 10) with a graticule. Usually these two values were the same but, if not, they were averaged to give a mean diameter. A range of nodule sizes was observed at each time and a median diameter was calculated. This median diameter was converted to a volume, assuming that the nodules were spheres, and the results are presented (fig 3) as the median nodule volume plotted as a function of time after injection of the cells. The time scales for the artificial and true metastases have been adjusted relative to one another by 2.5 days so that the data fall on the same line.

Drug Response of the Metastases

Treatment in vivo with a single dose of CY was initiated over a wide range of sizes for the artificial metastases, and, on the basis of the results, over a range of about 1–35 mm^3 for the true metastases. The effectiveness of the treatment was assessed using two criteria: (*a*) the fraction of mice surviving 60 days after treatment without evidence of tumor (survivor); and (*b*) the fraction of mice dying with evidence of metastatic tumor regrowth (recurrence). Details of how these criteria have been applied are given in the Materials and Methods section.

The combined results from a number of experiments in which a single dose of 300 mg/kg of CY was given to the mice are shown in figure 1A for the artificial metastases, and in figure 1B for the true metastases. The percentage of survivors or recurrences is shown on the two vertical axes of figures 1A and B while the time after the injection of the cells and the corresponding median nodule volume are shown on the horizontal axes. The line drawn to represent the recurrence data in figure 1A has been derived from the Poisson distribution on the basis of the assumption that 63% recurrences will be obtained when, on the average, one tumor cell survives per mouse; it is fitted to the data at the level of 10% recurrences (equivalent to an average survival of 0.1 tumor cell/mouse). For the survival data a line of the same shape has been assumed and it

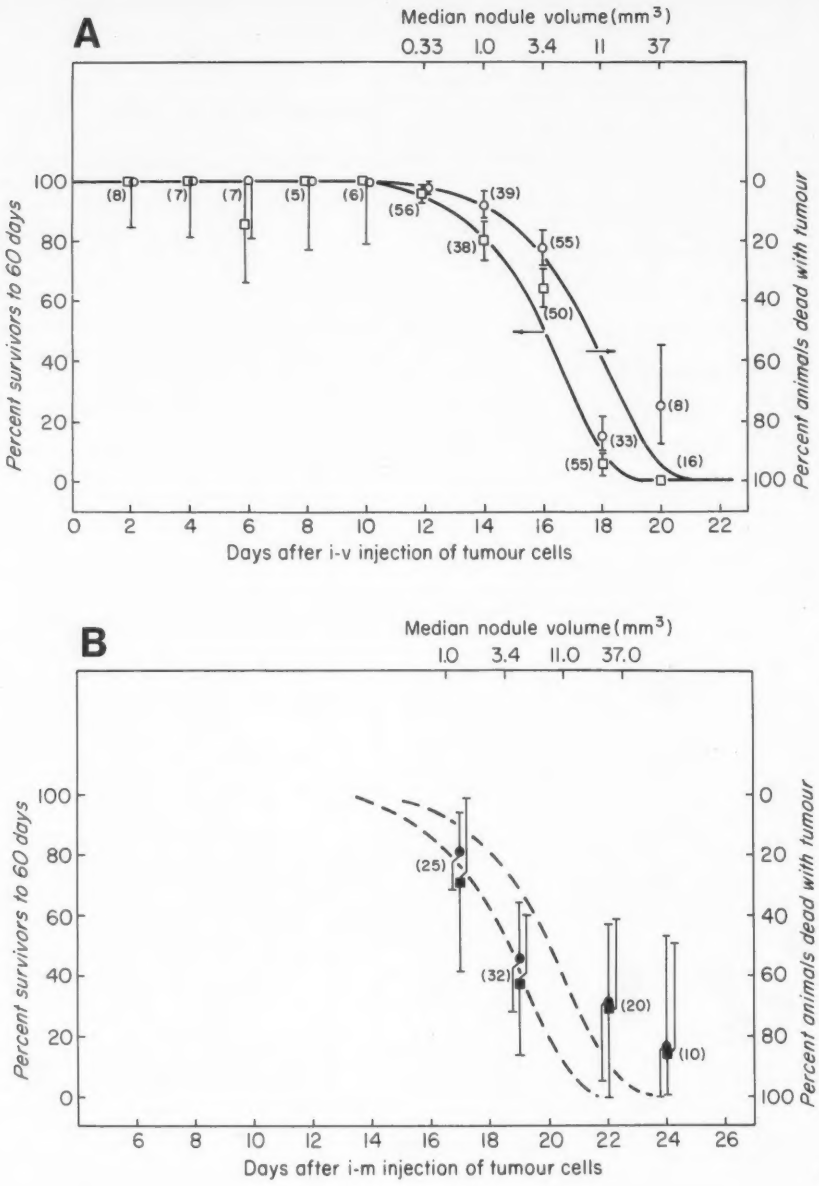
A
Median nodule volume (mm³)
0.33 1.0 3.4 11 37
Percent survivors to 60 days
Percent animals dead with tumour
Days after i-v injection of tumour cells
(8) (7) (7) (5) (6) (56) (39) (38) (55) (50) (8) (33) (55) (16)
B
Median nodule volume (mm³)
1.0 3.4 11.0 37.0
Percent survivors to 60 days
Percent animals dead with tumour
Days after i-m injection of tumour cells
(25) (32) (20) (10)

FIGURE 1.—*A.* % of animals surviving to 60 days (□) after CY treatment plotted as a function of tumor size for artificial metastases; also shown is % of animals dying with tumor (○). Tumor size has been related to the time scale using fig 3. Bars = SEs. Nos. in parentheses indicate the No. of animals contributing to each point; where there is only 1 No. it refers to both points at that time. The fitting of the lines is discussed in the text. *B.* Same as *A* except that the results are for true metastases. ----- are taken from *A*; Nos. in parentheses indicate the No. of mice initially treated at each time point (approximately half of these mice would have had true metastases).

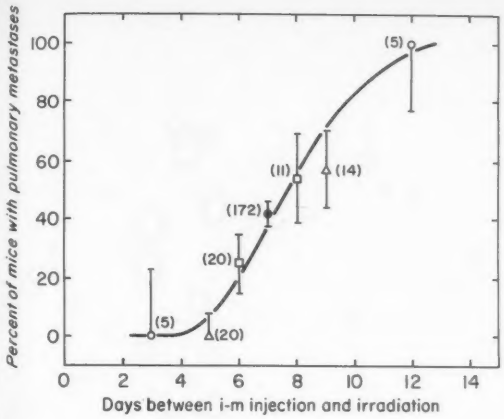

FIGURE 2.—% of mice which developed pulmonary metastases plotted as a function of time between the im injection of tumor cells and irradiation to ablate the primary tumor. ○, □, △ = observations made on 3 different groups of mice with SEs (calculated from the binomial distribution) shown; ● = mean of a No. of independent determinations with the SE calculated from the range of these observations (ie, assuming the individual means to be normally distributed); Nos. in parentheses indicate the actual No. of mice contributing to each point. The line through the data is drawn by eye.

has been moved on the time axis to fit the data points. These two lines are also shown in figure 1B.

Cell Kinetics of Lung Nodules

The cell cycle parameters of the cells growing in the true and artificial metastases were investigated, in the same range of nodule sizes in which the cures with CY were observed, by determining percent labeled mitoses curves and initial labeling indices. The results of the study showed very little difference in the cell cycle parameters for any size of nodule examined, regardless of whether they were true or artificial metastases. Two representative percent labeled mitoses curves are shown in figure 4 and the same line has been drawn through each set of points to indicate the similarity of the data. The results have been analyzed using the computer model of Steel and Hanes (9) and the cell cycle parameters so derived are shown in table 1.

DISCUSSION

The results shown in figure 1 indicate how rapidly the Lewis lung tumor, transplanted into an im site, will metastasize to the lung tissue. The results agree well with those reported by Humphreys and Karrer (1) who transplanted their tumors by im

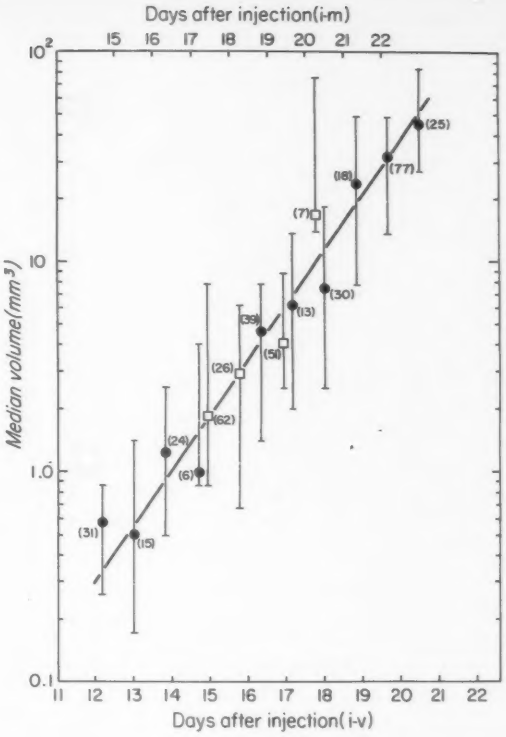

FIGURE 3.—Growth curve for the lung metastases. ● = artificial metastases and refers to the lower time scale; □ = true metastases and refers to the upper time scale. These 2 time scales have been adjusted relative to one another so that the data fit on a single line. The errors shown on this figure are the quartile values derived from the distributions of tumor sizes at each time point. Nos. in parentheses are the No. of nodules contributing to each point.

injection of a cell suspension (number of cells injected was not given but from other data the authors' estimate is 1–2 × 10^6), a similar technique to the one reported here. However, Mayo et al (2) reported a much higher incidence of metastases with 100% at 6 days after transplanting tumor fragments sc with a trocar.

Figure 3 indicates that over the range of sizes from 0.4 to 40 mm^3 the lung metastases were growing at a constant rate with a doubling time of about 30 hours. This was true for both artificial and true metastases. Assuming that the artificial metastases seed within a few hours of the iv injection, it can be seen from the relative displacement of the time scales in figure 3 that the true metastases were growing as if they were seeded 2.5 days after the im

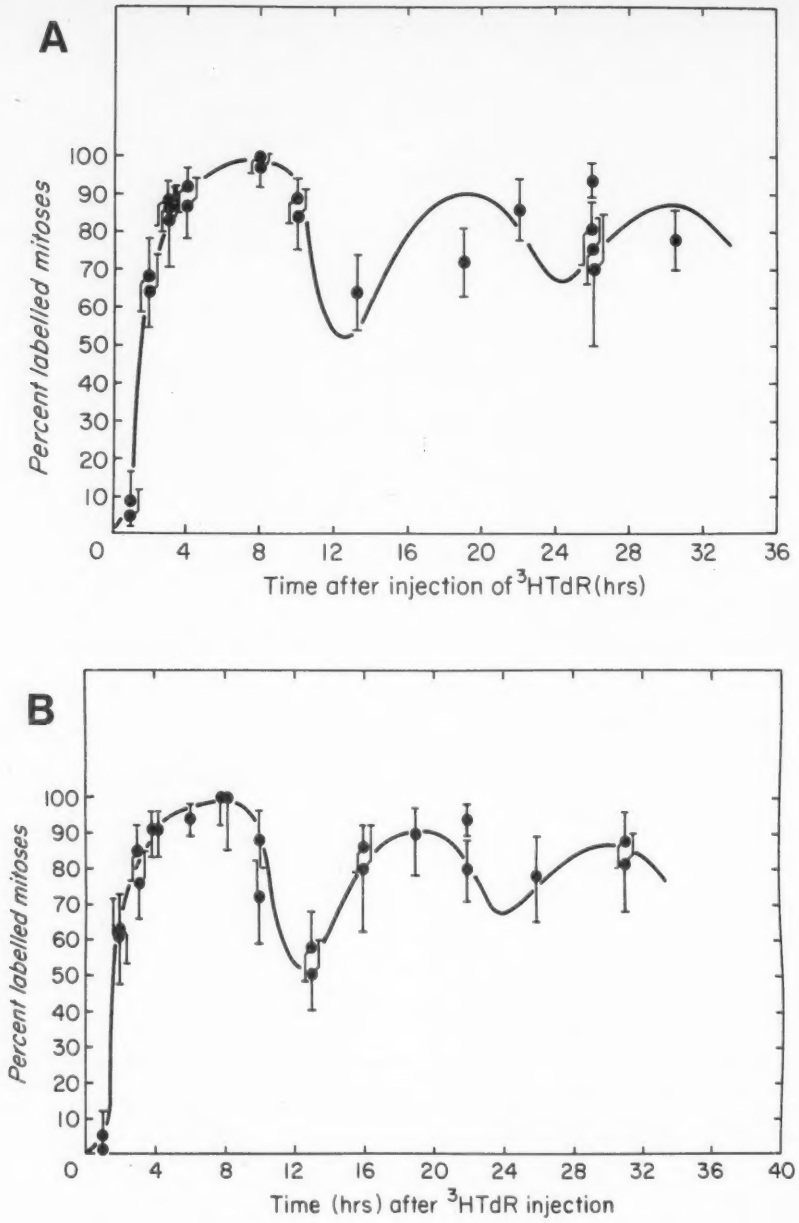

FIGURE 4.—*A*. % labeled mitoses curve for artificial metastases at 16 days after iv injection of the cells. ● = one nodule and the 95% confidence limits derived from the binomial distribution. *B*. % labeled mitoses curve for true metastases at 19 days after im injection of the tumor cells (approximately equivalent on the basis of size to Day 16 for artificial metastases). ● = one nodule and 95% confidence limits derived from the binomial distribution.

TABLE 1.—Lewis lung tumor cell cycle parameters

No. of days	Type of metastasis	Labeling index (%)	Cell cycle parameters*				Growth fraction (%)
			T_{G_2}	T_S	T_{G_1}	T_C	
12	Artificial	48.2	1.2 (1.7; 1.8)	9.3 (9.4; 1.5)	0.1 (0.5; 2.3)	11.2	57
14	Artificial	51.0	1.6 (1.8; 0.7)	9.0 (9.2; 1.7)	0 (0.4; 5.1)	10.9	61
16	Artificial	54.2	1.3 (2.0; 2.1)	10.1 (10.1; 0.9)	0.5 (1.6; 5.1)	12.6	68
18	Artificial	56.9	1.1 (1.8; 2.3)	10.1 (10.2; 1.1)	0.1 (0.6; 4.5)	11.8	67
19	True	48.5	1.5 (2.0; 1.7)	9.8 (9.9; 1.0)	0.1 (0.7; 2.9)	12.0	60

*Derived using the computer model described by Steel and Hanes (ref 9). Values given are the median durations in hrs; also indicated are the parameters of the log-normal distribution of phase durations given in the form (mean; standard deviation).

implant. The results of figure 2, however, suggest that cells capable of forming metastases were not seeded into the lung until 4–5 days after the im implant. Since there is evidence that the artificial metastases seed from a single viable cell (4), this discrepancy in the results suggests either that the true metastases seed from a clump of cells or that there is some factor which allows them to grow faster initially than the artificial metastases.

Figure 1A shows that for the treatment of artificial pulmonary metastases with a single large dose of CY, the data for both recurrence and survival are reasonably well fitted by curves derived using the Poisson distribution. This is in contrast with the results of Mayo et al (2) who found that with artificial metastases of the Lewis lung tumor there was a wide spread of time (and probably size) over which they could be cured with CY. All these times, however, were earlier than those observed in our experiments. This may be because Mayo et al (2) were apparently seeding a large number of artificial metastases per mouse whereas our experiments have been done with small numbers of metastases per mouse. The difference in the two curves (fig 1A) for the survival of the mice and for the rate of tumor recurrence becomes apparent only at ≥ 14 days after the injection of the tumor cells, which suggests that mice bearing advanced metastases at the time of treatment were more sensitive to the toxic effects of CY than mice with early metastases. This is in agreement with the results of DeWys (10).

For the true metastases (fig 1B) the results are compared with the lines representing the results for the artificial metastases. Lines of a much shallower slope would give a better fit to the data points. More mice survived when treatment was given at 22 and 24 days after the im implant than would have been expected on the basis of the results with artificial metastases. This difference could be due to the fact that, in groups of mice with a given mean number of true metastases, there was a higher percentage of mice with a small number of nodules (one or two) per mouse than was found in groups of mice with a similar mean number of artificial metastases.

The studies of the cell kinetics of the true and artificial metastases (fig 4) have not helped to elucidate the reason for the rapid change in curability of the tumors. It appears that, for all the sizes investigated, the tumors were growing very rapidly. CY is a drug which is not thought to be phase-specific, but there is evidence that it is proliferation-dependent (11–13); thus a change, eg, in the growth fraction of the metastases, could have explained the results of the cure experiments. As can be seen from table 1, however, there is no evidence of this, although, of course, there could be very small foci of slowly proliferating cells developing in the colonies which would not be detectable in the labeling studies. The histologic sections of the metastases have been examined for developing areas of necrosis and, although these areas become larger and more frequent as size increases, they are detectable even in some 12-day tumors. The percent labeled mitoses curves for the metastases are quite similar to those of Simpson-Herren et al (14), who studied Lewis lung tumor metastases growing in BDF_1 mice, except that their curves have a lower second peak. This is reflected in the prediction from the computer curve fitting that the cells in the metastases studied by them had a larger median intermitotic interval (15–16 hours) than the cells in the metastases investigated in this series of experiments (11–13 hours).

The finding that the results for the CY treatment of the artificial metastases (fig 1A) can be reasona-

bly well fitted by a Poisson distribution implies that the increase in cell number is sufficient to explain the change in the response as a function of size, and that no change in the drug sensitivity of the cells need be postulated. Steel and Adams (5) have shown that the cells in im Lewis lung tumors of the subline used in these experiments would be reduced to a survival level of about 10^{-6} after a dose of 300 mg/kg of CY, and their results suggest that small pulmonary tumors may be even more sensitive. Assuming the tumors contain about 5×10^8 cells/cc, then Day-14 artificial metastases would contain about 5×10^5 cells and it might be expected that 300 mg/kg of CY would reduce survival to the level of one cell or less and that cures would be observed since each mouse had only a few nodules. Also, initial studies with a dose of 150 mg/kg of CY appear to define a curve of very similar shape to the one observed for 300 mg/kg displaced to earlier times by about 6 days. This time difference is equivalent to about five volume doublings of the tumor and hence a reduction in volume by a factor of about 32. The results of Steel and Adams (5) indicate that a reduction in CY dose from 300 to 150 mg/kg should reduce the cell killing by a factor of about 100, which agrees reasonably well with this volume change. The interpretation is also supported by the experimental evidence that, if instead of a small number of metastases, the mice have 100–200 tumors per lung, then they cannot be cured by a single dose of CY (300 mg/kg), even on Day 12, which is similar to the results of Mayo et al (2) discussed earlier.

The suggestion that larger tumors are more difficult to cure than smaller ones is of course not new (*see* Schabel [15] for discussion in terms of micrometastases). However, in most cases in which this has been observed it is postulated that it is due, at least in part, to a difference in the growth kinetic parameters of small and large tumors. In the results presented here, when tumors which differed in size by a factor of 100 were studied, no changes in the growth kinetic parameters were detected and the increase in cell number in the larger tumors was sufficient, in itself, to explain their reduced curability.

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Antileukemic Effect of Homo-aza-steroidal Ester of [*p*-[Bis(2-chloroethyl)amino]phenyl]Acetic Acid [1,2,3]

Galen L. Wampler[4,*] and Panayotis Catsoulacos[5,6]

SUMMARY

The homo-aza-steroidal ester of [*p*-[bis(2-chloroethyl)amino]phenyl]acetic acid, 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17,-lactam *p*-bis(2-chloroethyl)aminophenylacetate (ASE), gives a > 50% increased lifespan over controls in the treatment of L1210 leukemia by the ip, sc, and oral routes of administration and a > 150% increased lifespan in the treatment of P388 leukemia by the ip route (the only route tested). Both a daily and a Day 1, 5, and 9 treatment schedule are essentially equally effective. In this study, ASE is compared to the parent compound phenesterin which is inactive in both of these tumor lines. To our knowledge ASE is the first steroidal alkylating agent to show activity in the L1210 leukemia.

[Cancer Treat Rep 61:37–41, 1977]

Recently, the attention of investigators has been increasingly attracted to a series of steroidal nitrogen mustards in which the active agent was linked to the steroid by a stable bond (1–7) or by a bond more easily cleaved by enzymatic action (8–14). Activity was found only in those instances in which the steroid and potential oncolytic agent were connected by ester or heterolytic linkage (13).

In view of the importance of steroids containing alkylating agents, it was decided to use modified steroids, such as lactams and lactones, as a biologically acceptable platform of transporting the alkylating agent to the tumor site in a rather specific manner (15–18). The biologic action of lactams, characterized by the –C–NH–group, may be structurally specific and therefore more prolonged as the result of the multiple interactions of such a group with similar groups that exist in proteins and nucleic acids.

Our satisfactory results in two experimental tumors (16) and the findings of Wall et al (13) that steroidal esters of [*p*-[bis(2-chloroethyl)amino]phenyl]acetic acid were inactive in L1210 leukemia (according to the criteria of the National Cancer Institute [NCI]) prompted us to study the 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17,-lactam *p*-bis(2-chloroethyl)aminophenylacetate (ASE) in two leukemias and compare the results with the extensively studied phenesterin.[7] The formulae of these two compounds are shown in figure 1.

MATERIALS AND METHODS

Phenesterin and ASE, synthesized by previously described methods (8,15), were dissolved in ethanol in a concentration of 10 mg/ml. A small amount of Tween 80 was added (equal to 0.1% of the final suspension), and then saline was added to make a final concentration of drug of 1 mg/ml. This produced a stable milky suspension which was freshly

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[2] Supported by the D. Harvey Marler Memorial Research Fund.

[3] [*p*-[Bis(2-chloroethyl)amino]phenyl]acetic acid: NSC-71964; CAS reg. No. 10477-72-2.

[4] Division of Medical Oncology, Virginia Commonwealth University, Richmond.

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[6] We wish to thank Juanita Coon and Ersell Dortch for their technical assistance with the animal experiments, and Brenda Newcomb for typing the manuscript.

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[7] Phenesterin: NSC-104469; CAS reg. No. 3546-10-9; cholesterol, [*p*[bis(2-chloroethyl)amino]phenyl]-, acetate.

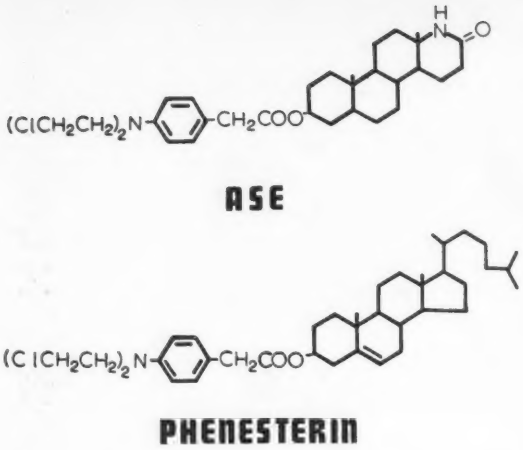

FIGURE 1.—Comparative chemical structures of ASE and phenesterin.

made for each separate experiment from the stock chemicals.

Drug in doses of 0.13–0.9 ml (6.7–45 mg/kg/injection) was administered by the ip, sc, or oral route in several schedules: Days 1–8; Days 1, 5, and 9; or Day 1 only.

L1210 leukemia was maintained in our laboratory by weekly ip passage of 10^5 L1210 cells in DBA/2 mice. P388 leukemia was maintained by weekly ip passage of 10^6 P388 cells into DBA/2 mice. C57BL/6 × DBA/2 ($B6D2F_1$) female mice, average weight, 20 g each, were used for the test animals in both cases. L1210 (10^5) or P388 (10^6) cells were inoculated ip into each test animal; the lifespan was recorded. For all experiments, mice (uniform as to sex and age) were kept in groups of eight in an air-conditioned, light-controlled environment.

RESULTS

Phenesterin (the parent compound) was inactive in these two tumor systems. In none of the experiments was the increased lifespan (ILS) of treated

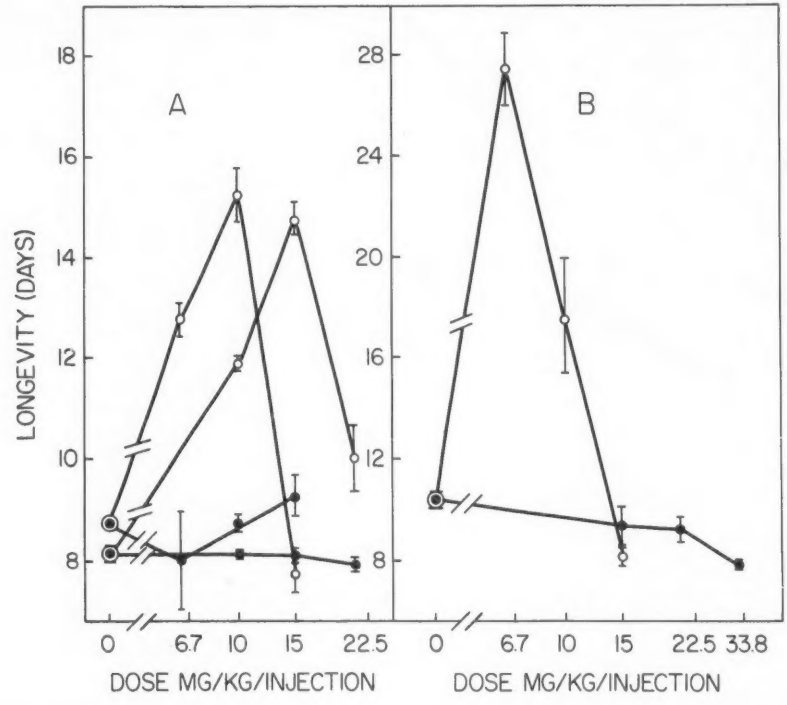

FIGURE 2.—Dose-response curves for ASE (○) and phenesterin (●) in (A) L1210 leukemia and (B) P388 leukemia for ip drug treatment on a Day 1–8 schedule. Points represent mean lifespan ± SE of 8 female $B6D2F_1$ mice; each curve represents the dose response for 1 experiment.

TABLE 1.—Activity of phenesterin and ASE in L1210 leukemia*

Compound (route of administration)	Schedule†					
	Days 1–8		Days 1,5,9		Day 1 only	
	Dose	ILS	Dose	ILS	Dose	ILS
Phenesterin (ip)	6.7	−7	20	0	20	1.5
	10	0,0	30	0	30	−1.5
	15	0,−1.5	45	−3	45	−3
	22.5	1.5,6				
ASE (ip)	6.7	46	20	57	20	16
	10	46,74	30	60	30	7
	15	82,−11	45	51	45	−74
	22.5	23,−66				
Phenesterin (sc)	10	−3	20	1.5		
	15	−1.5	30	1.5		
	22.5	−6	45	1.5		
ASE (sc)	10	38	20	38		
	15	56	30	38		
	22.5	10	45	−31		
Phenesterin (oral)	10	−4	20	−3		
	15	16	30	3		
	22.5	0	45	−1.5		
ASE (oral)	10	40	20	59		
	15	46	30	46		
	22.5	−31	45	−18		

*L1210 cells (10^5) were injected ip on Day 0; treatment was started 24 hrs later using 8 female B6D2F$_1$ mice/group.
†Dose, mg/kg/injection; % ILS calculated as follows: lifespan (treated) − lifespan (controls) × 100/lifespan (controls).

TABLE 2.—Activity of phenesterin and ASE in P388 leukemia*

Compound (route of administration)	Schedule†			
	Days 1–8		Days 1,5,9	
	Dose	ILS	Dose	ILS
Phenesterin (ip)	15	−11		
	22.5	−12		
	33.8	−25		
ASE (ip)	6.7	161	15	186‡
	10	68	22.5	79
	15	−22		

*P388 cells (10^6) were injected ip on Day 0; treatment was started 24 hrs later using 8 female B6D2F$_1$ mice/group.
†Dose, mg/kg/injection; ILS, %.
‡1 survivor in this group was not included in the calculation.

animals 16% over controls. A ≥ 25% ILS is usually required as a minimum for activity. This corroborates the findings of the NCI for phenesterin in L1210 leukemia (10). In contrast, ASE produced maximal activity of 82%, 56%, and 46% ILS by the ip, sc, and oral routes respectively, on a daily treatment schedule in L1210 leukemia (*see* table 1) and 60%, 38%, and 59% ILS by the ip, sc, and oral routes respectively, using the Day 1, 5, and 9 treatment schedule. Use of the drug in a single injection on Day 1 gave inferior results and this treatment schedule was abandoned.

Maximal activity of 161% ILS on the daily schedule and 186% ILS (with one survivor) on the Day 1, 5, and 9 schedule were obtained in P388 leukemia (table 2).

Optimum doses for ASE in L1210 leukemia by all routes of administration were essentially the same (10–15 mg/kg/injection on the daily schedule [80–120 mg/kg total dose] and 20–30 mg/kg/injection on the Day 1, 5, and 9 schedule [60–90 mg/kg total dose]). In P388 leukemia, the optimum dose appears to be slightly less (fig 2). For phenesterin, no optimum dose could be assigned in view of the lack of activity. However, phenesterin appeared to be slightly less toxic than ASE by weight based on drug deaths seen at the higher dose levels of both compounds. Drug-treated non-tumored animals were not utilized but toxicity was judged to be present when, at a given dose level, more than one animal died before any control animal deaths occurred.

Weight loss with ASE treatment at the optimum dose averaged 24%, 21%, and 23% of body weight on Day 8 by the ip, sc, and oral routes respectively, in L1210 leukemia, and 15% by Day 8 in P388 leukemia by the ip route (as previously noted, optimum doses were lower in P388 leukemia).

DISCUSSION

ASE is active in the treatment of the L1210 and P388 murine leukemias. Treatment results are clearly superior to those obtained with phenesterin, the most nearly related drug that has been in clinical trial,* and are equal to or better than many clinically useful drugs in these two screening tumor systems (19). All of the steroidal alkylating agents studied by the NCI and reported by Wall and coworkers (13) were inactive in L1210 leukemia, and, to our knowledge, this is the first such compound with activity in this important screening tumor. While a number of alkylating agents (eg, cyclophosphamide) are more active in L1210 leukemia, as suggested in an earlier paper (20), a direct comparison of activity for two drugs having different schedule dependencies may not be appropriate.

Furthermore, the direct comparison of ASE with classic alkylating agents ignores the possible benefits provided by the steroidal moiety and easily cleaved ester bond, both of which provide theoretic variations in mechanisms of action which may influence the relative activity in the mouse leukemias versus, for example, hormonally active human solid tumors. This is supported by a recent success reported for estramustine phosphate in advanced prostate carcinoma (21).

In an Eastern Cooperative Oncology Group phase II study, phenesterin gave a 7.5% response rate in the treatment of variety of human solid tumors, most of which had been previously treated with chemotherapy. A 2.5% complete response rate was seen including two breast carcinomas, two ovarian carcinomas, and one uterine carcinoma.* Since ASE is active in the treatment of L1210 leukemia (a tumor known for its predictive capacity for activity in human malignancies) and phenesterin is inactive, further development of ASE with an aim toward human trials is warranted.

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Treatment of Chronic Myeloid Leukemia With Repeated Single Doses of Busulfan [1,2]

J. R. Sullivan,* T. H. Hurley, and J. H. Bolton [3]

SUMMARY

Repeated single doses of busulfan (50–200 mg) were given over 1–2 days to 17 patients with chronic myeloid leukemia. The mean survival time was 137 weeks. In this small trial the drug was shown to be safe in most but not all patients and as effective as continuous busulfan.

[Cancer Treat Rep 61:43–45, 1977]

The established method of administration of busulfan is a daily dose of 4–6 mg given for several weeks. The drug is stopped when the wbc count reaches about 20,000/mm^3 since experience has shown that the wbc count may continue to fall for several weeks.

Patients must be carefully supervised during treatment to avoid overdosage and fatal marrow aplasia. In practice, such supervision may be difficult in those patients who do not appreciate the hazards of their treatment regimen. Practical difficulties may arise with patients in remote areas. Some of these difficulties can be overcome by using busulfan as a single large dose. The safety and effectiveness of such treatment was suggested by experience with single-dose regimens in chronic lymphatic leukemia (1).

PATIENTS AND METHODS

Seventeen patients aged 29–80 years (mean, 55 years) with chronic myeloid leukemia were treated with repeated single-dose busulfan from 1958 to 1975. The diagnosis in all patients was based upon a total wbc count > 30,000/mm^3 with a typical spectrum of granulocytic immaturity in the peripheral blood but with < 30% myeloblasts and promyelocytes. Bone marrow aspirations showed characteristic granulocytic hyperplasia. A Philadelphia (Ph^1) chromosome was present in 11 patients. In three patients diagnosis was made prior to the discovery of the Ph^1 chromosome. Satisfactory chromosome analysis was not possible in the remaining three patients for technical reasons. In each patient the clinical features including splenomegaly were typical.

The doses of busulfan given were 50, 100, or 200 mg taken in divided doses over 1–2 days as 2-mg tablets. Some patients experienced nausea because of the large number of tablets ingested but no vomiting occurred. Hemoglobin, total wbc count, differential wbc count, and platelet count were done every 2 weeks for 6 weeks following treatment.

RESULTS

Repeated single doses of busulfan were used on 69 occasions in the 17 patients over a total of 40 patient years. The mean survival time was 137 weeks (range, 30–337 weeks) from diagnosis and 104 weeks (range, 5–332 weeks) from treatment with single-dose busulfan (table 1). The mean total dose of busulfan given as single doses was 406 mg (range, 100–1200 mg). The mean total continuous busulfan dose was 308 mg (range, 144–742 mg). The mean times between courses of repeated single-dose busulfan are shown in table 2.

The nadir of the total wbc and platelet counts occurred within 2–6 weeks after treatment. The lowest wbc count was 900/mm^3 in patient 10 following a 100-mg dose of busulfan. There were no clinical sequelae and the wbc count returned to normal within 4 weeks. The lowest platelet count was 7000/

[1]Received July 13, 1976; revised Nov 30, 1976; accepted Dec 14, 1976.

[2]Busulfan: NSC-750; CAS reg. No. 55–98–1; 1,4-butanediol, dimethanesulfonate.

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TABLE 1.—Patient data

Patient No.	Age at diagnosis (yrs)	Ph¹ chromosome	Survival from diagnosis (wks)	Survival from single dose of busulfan	Continuous busulfan prior to single dose	No. of single doses of busulfan (mg)			Initial hemoglobin (g/100 ml)	Initial total wbc count (cells/mm³)	Initial platelet count (cells/mm³)	Range of total wbc after single dose of busulfan		Range of platelet count after single dose of busulfan	
						50	100	200				Lowest (cells/mm³)	Highest (cells/mm³)	Lowest (platelets/mm³)	Highest (platelets/mm³)
1	37	+	104	104	–	—	3	1	11.2	80,000	—	6000	77,000	80,000	100,000
2	47	+	111	86	+	1	7	—	12.0	340,000	800,000	13,000	102,000	92,000	650,000
3	58	+	337	41	+	—	3	—	12.1	200,000	300,000	4000	29,000	200,000	330,000
4	66	+	46	46	–	1	3	—	11.4	290,000	170,000	31,000	78,000	175,000	325,000
5	37	+	118	63	+	—	4	—	9.7	206,000	184,000	1200	7000	7000	110,000
6	80	+	134	122	+	—	9	—	8.2	340,000	200,000	6900	11,000	142,000	500,000
7	52	+	30	5	+	—	1	—	6.0	100,000	170,000	6400	6400	90,000	90,000
8*	49	–	164	164	–	2	5	—	13.3	45,000	1,000,000	5000	26,000	300,000	620,000
9	38	–	120	99	+	—	2	2	9.0	960,000	300,000	28,000	180,000	38,000	240,000
10*	54	–	332	332	–	—	3	—	9.3	90,000	400,000	900	11,000	20,000	400,000
11*	73	–	64	64	–	—	3	—	12.5	150,000	1,100,000	15,000	50,000	860,000	2,900,000
12	29	–	288	288	–	—	2	5	11.6	178,000	254,000	4500	28,000	190,000	230,000
13	62	–	116	11	+	—	1	—	8.2	160,000	150,000	9000	9000	60,000	60,000
14†	71	+	120	82	+	—	4	—	7.1	324,000	270,000	2100	2200	70,000	120,000
15	35	+	105	105	–	—	7	—	8.0	130,000	6,000,000	2300	16,000	30,000	500,000
16†	50	+	85	85	–	—	10	—	10.2	230,000	85,000	20,500	41,000	200,000	280,000
17†	62	+	71	71	–	—	6	—	9.3	220,000	145,000	29,000	100,000	175,000	225,000

*Diagnosis made prior to 1960.
†Patient still alive (Apr 9, 1976).

TABLE 2.—Mean (± SD) interval between treatment with single doses of busulfan of varying size

Dose (mg)	Mean interval between doses (wks)	No. of single doses given
50	15 ± 9	5
100	16 ± 11	69
200	29 ± 15	8

mm^3 in patient 5 following a 100-mg dose of busulfan. This patient was considered to have blastic transformation. Busulfan had no effect clinically and the patient died 2 months later. The total wbc count fell to $< 10{,}000/mm^3$ on 12 occasions and the platelet count to $< 100{,}000/mm^3$ on seven occasions.

Spleen size decreased with treatment except in those instances in which blastic transformation had supervened. In these cases busulfan had little or no effect on the progress of the disease, although in several instances death occurred within several weeks of this treatment. Fourteen of the patients have died: two of unrelated causes, two following splenectomy, one of gastrointestinal hemorrhage with a normal platelet count, one of generalized herpes simplex, and the remainder of acute transformation.

DISCUSSION

Two forms of treatment are currently used for chronic myeloid leukemia: splenic irradiation and continuous busulfan. With splenic irradiation a median survival of 8–37 months has been demonstrated. Daily-dose busulfan can result in a median survival time of 31–45 months (2–4). The mean survival time of 137 weeks in this study is similar and consistent with the accepted view that treatment in chronic myeloid leukemia can improve the quality of life but that survival time is not significantly prolonged.

The convenience of the single-dose method of administration is its principal advantage. This method of treatment also reduces the risk of inadvertent overdosage which may occur with continuous therapy, particularly in old, confused, or demented patients.

Busulfan seems to affect the multipotential (5) precursor stem cell so that the full effects of a single large dose of busulfan take up to 6 weeks to become evident. Repeating busulfan during this time would increase the risk of profound marrow depression and for this reason we have avoided re-treatment at intervals of < 6 weeks.

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Evaluation of Single-Drug Versus Multiple-Drug Chemotherapy in the Treatment of Advanced Breast Cancer [1]

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SUMMARY

Fifty-one postmenopausal patients with advanced breast cancer were randomized to receive either cyclophosphamide given continuously or cyclophosphamide given continuously with methotrexate, 5-fluorouracil, vincristine, and prednisone given intermittently. The results with the multiple-drug therapy were significantly better than with the single-drug therapy, both with regard to percentage (63% versus 25%) and median duration (400 versus 210 days) of objective response. Toxicity was moderately increased with the combination as compared to the single-drug treatment.

[Cancer Treat Rep 61:47–50, 1977]

During recent years single-drug treatment of advanced breast cancer has to an increasing extent been substituted by different multiple-drug combinations. The most frequently used agents have been cyclophosphamide, methotrexate, 5-fluorouracil, and vincristine.[3] The response rates obtained with each of these drugs differ very much in different studies, the range for cyclophosphamide being 10%–62%, for methotrexate 27%–54%, for 5-fluorouracil 13%–40%, and for vincristine 13%–40% (4). By combining these four drugs and adding prednisone,[4] Cooper obtained responses in 90% of the patients treated (2), but in later similar studies the response rates have been close to 60% (3–5).

These results might indicate that the five-drug combination is more effective than the single-drug treatment but many conditions render a direct comparison impossible, especially differences in patient selection, duration of treatment, and definition of response.

The present study is a controlled comparison between single-drug treatment with cyclophosphamide and multiple-drug treatment with cyclophosphamide, methotrexate, 5-fluorouracil, vincristine, and prednisone in postmenopausal patients with advanced breast cancer.

PATIENTS AND METHODS

Fifty-five consecutive patients admitted to the Copenhagen University Hospitals Gentofte and Glostrup with advanced breast cancer entered the trial. Eligibility requirements for the study included: (*a*) The patients should be postmenopausal and < 75 years of age. The postmenopausal state was defined by at least 12 months of normal menostasis. Patients who had previously had hysterectomies were considered postmenopausal from the age of 51 years. (*b*) The metastatic disease should be verified histologically. (*c*) There should be measurable disease. (*d*) The patient should not have received prior chemotherapy. (*e*) There should be no CNS involvement of the disease. (*f*) The patients should have given their informed consent.

The patients were randomly allocated (using a stochastic array of numbers, closed-envelope system) to one of two treatment groups: A = single-drug group, cyclophosphamide alone; B = multiple-

[1] Received July 22, 1976; revised Oct 22, 1976; accepted Nov 9, 1976.

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[3] Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

[4] Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-.

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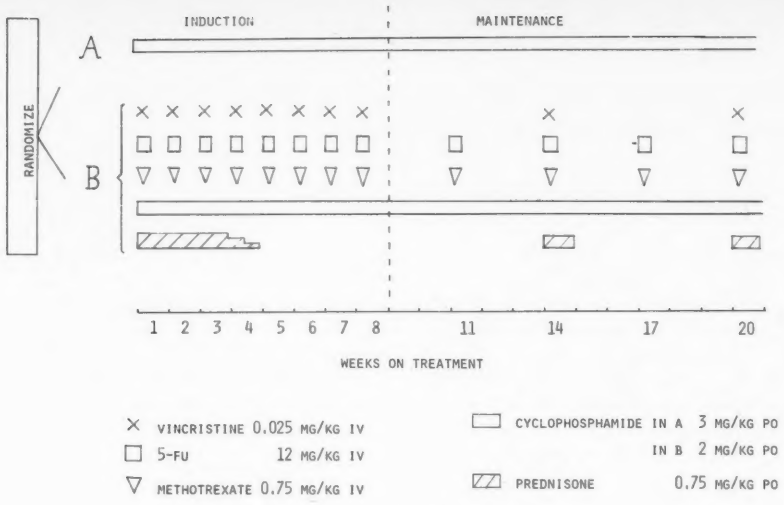

FIGURE 1.—Single-drug vs multiple-drug chemotherapy in metastatic breast cancer: treatment regimens.

drug group, combination of cyclophosphamide, methotrexate, 5-fluorouracil, vincristine, and prednisone. The complete regimens are shown in figure 1.

Treatment was continued until progressive disease or until relapse after previous remission. If complete remission occurred treatment was discontinued after 2 years. If toxicity occurred doses of methotrexate, 5-fluorouracil, and cyclophosphamide were modified according to the following leukocyte and thrombocyte values (cells/μl) respectively: > 3000 and > 100,000, 100% of dose; 1000–3000 and 50,000–100,000, 50% of dose; < 1000 and < 50,000, no drug. Equal bone marrow toxicity was aimed at in the two treatment groups.

In the case of neurologic toxicity of grades 1, 2, and 3, doses of vincristine were 100%, 50%, and 0 respectively.

At the beginning of the treatment patients were assessed by physical examination, chest roentgenogram, skeletal survey, laboratory tests, and estimation of the performance status (Karnofsky scale). All visible and palpable lesions were measured to provide a baseline for subsequent examinations. These were repeated after completion of the induction treatment and thereafter at 1–3-month intervals.

None of the patients had liver metastases only. Liver scintiscans were not done systematically and the scans were not used for evaluation of the effect of treatment. Seven and nine patients, for regimens A and B respectively, had bone metastases only.

The definitions of objective response were as follows: *complete remission* (CR) = complete disappearance of all measurable lesions; *partial remission* (PR) = 50%–99% reduction of measurable lesions and/or definite recalcification of bone lesions; *no change* (NC) = < 50% reduction of measurable lesions and/or no visible change of bone lesions.

RESULTS

The patient characteristics are shown in table 1. Twenty-seven patients were randomized to the single-drug therapy and 28 to therapy with cyclophosphamide, methotrexate, 5-fluorouracil, vincristine, and prednisone. Four patients died with rapidly progressive disease within 4 weeks after initiation of the treatment and were therefore considered nonevaluable. Thus, 24 and 27 patients, respectively, could be evaluated for regimens A and B. The patients randomized to each of the two regimens were comparable with regard to age, length of disease-free interval, previous endocrine therapy, performance status, and dominant disease site. At the time of analysis of the results 12 months had passed since entry of the last patient.

The results of therapy are shown in table 2. Sixty-three percent obtained remission (PR + CR) with the multiple-drug therapy compared to only 25% with the single-drug therapy. This difference is highly significant ($P < 0.01$, Mann-Whitney rank sum test). A similar significant difference was obtained with regard to median duration of remission: 400 versus 210 days for regimens A and B respec-

TABLE 1.—Patient data: single-drug vs multiple-drug chemotherapy

	Single drug		Multiple drug	
No. of patients entered	27		28	
No. of patients evaluable	24		27	
Median age in yrs (range)	61	(48–76)	62	(44–70)
Median disease-free interval in yrs (range)	3½	(0–11)	4	(0–10)
No. with previous endocrine therapy (%)	3	(13)	5	(19)
Median performance score (Karnofsky) (range)	60	(30–90)	50	(30–90)
No. (%) with dominant disease site in—				
Bones	9	(38)	11	(41)
Soft tissue	8	(33)	9	(33)
Viscera	7	(29)	7	(26)

TABLE 2.—Results of single-drug vs multiple-drug chemotherapy

	Single drug		Multiple drug	
Results*	No.	%	No.	%
PD	10	41	4	15
NC	8	34	6	22
PR	5	21	12	44
CR	1	4	5	19
PR + CR	6	25	17	63
Median duration of CR + PR in days (range)	210 (150–540)		400 (150–2300+)	

*PD = progressive disease; NC = no change; PR = partial remission; CR = complete remission.

tively. In the multiple-drug group the duration of remission was 195–2300+ days (median, 495 days) among those who achieved CR and 150–1050 days (median, 330 days) among those achieving PR.

All patients in the single-drug group have died. In the multiple-drug group four patients are still alive, two with PR (330+ and 900+ days) and two with CR (495+ and 2300+ days).

Survival could not be compared between the two groups because all the patients with progressive disease received secondary regimens as part of new phase I or II studies.

The toxicity of the treatment is shown in tables 3 and 4. As mentioned above we aimed at equal bone marrow toxicity in the two groups. However, as shown in table 3, the bone marrow depression was somewhat more pronounced in the multiple-drug group. The other side effects, loss of hair and stomatitis, also occurred with higher frequency in the multiple-drug group, whereas the gastrointestinal toxicity was equally pronounced with the two therapies. Neurologic side effects, as expected, occurred only in the group receiving vincristine. None of the deaths could be ascribed to toxicity.

TABLE 3.—No. of patients with hematologic toxicity: single-drug vs multiple-drug chemotherapy

		No. of patients	
	Cells × $10^3/\mu l$	Single drug	Multiple drug
Leukocytes	> 3.0	12	7
	1.0–3.0	11	16
	< 1.0	1	4
Thrombocytes	> 100	13	9
	50–100	10	15
	< 50	1	3

TABLE 4.—No. of patients with toxicity: single-drug vs multiple-drug chemotherapy

Toxic effect	Degree*	Single drug	Multiple drug
Neurologic	0	0	6
	1	0	12
	2	0	8
	3	0	1
Gastrointestinal	0	12	10
	1	6	8
	2	6	8
	3	0	1
Stomatitis	0	23	10
	1	1	6
	2	0	8
	3	0	3
Loss of hair	0	12	0
	1	4	6
	2	5	11
	3	3	10

*0 = none; 1 = mild; 2 = moderate; 3 = severe.

DISCUSSION

Several studies from recent years have suggested that multiple drugs given in combination are more effective than the agents used singly (6,7). However, the controlled trials with single-drug treatment versus multiple-drug treatment in advanced breast cancer published so far have not convincingly demonstrated the superiority of the latter with regard to rate and duration of objective response.

Thus, 5-fluorouracil has been shown to be as effective as a five-drug combination of 5-fluorouracil, methotrexate, vincristine, prednisone, and chlorambucil (8). Similarly, Rubens, Knight, and Hayward (9), in a study comparing cyclophosphamide alone with a combination of cyclophosphamide, methotrexate, 5-fluorouracil, and vinblastine, found the number and duration of objective responses to be only insignificantly greater in patients receiving the combination. Surprisingly, in this study the combination was easier to manage and produced less toxicity.

The Eastern Cooperative Oncology Group has demonstrated the superiority of a combination of cyclophosphamide, methotrexate, and 5-fluorouracil over melphalan alone (10). However, the response rate of 20% with melphalan in this trial was inferior to the response expected with any of the other agents if they had been used singly (1). Therefore, although the results are convincing, the trial did not clearly prove that combinations are more effective than single agents.

The present study has demonstrated that a five-drug combination of cyclophosphamide, methotrexate, 5-fluorouracil, vincristine, and prednisone is superior to cyclophosphamide alone with regard to response rate and duration of response. The toxicity was moderately increased in the combination group as compared to the cyclophosphamide group but not to an extent which could invalidate the superiority of the combination treatment with regard to its effectiveness.

Extensive studies are still needed to finally elucidate the value of single-drug versus multiple-drug treatment, including multiple-drug treatment with different combinations and a different number of drugs.

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Phase II Study of 5-Azacytidine in Solid Tumors [1,2,3,4]

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SUMMARY

A phase II study utilizing 5-azacytidine in the treatment of patients with solid tumors was carried out by the Southwest Oncology Group (SWOG-7208). Of 214 patients entered in the study 191 were eligible and 167 were evaluable. While initially they received 225 mg/m² iv on Days 1–5 every 3 weeks, because of toxicity the dose was subsequently reduced to 175 mg/m² and later to 150 mg/m². Five partial regressions, 2.6% of the eligible patients and 3% of the evaluable patients, lasting from 28 to 77 days were observed. Sixteen patients, 8.4% of the eligible patients and 9.6% of the evaluable patients, had no significant change in their disease for 39–255 days. The major toxicities were myelosuppressive and gastrointestinal with 13 deaths attributable to drug toxicity: 11 due to sepsis and two due to cerebral hemorrhage. 5-Azacytidine induced few favorable responses; those that did occur usually were of poor quality and short duration and were associated with significant toxicity.

[Cancer Treat Rep 61:51–54, 1977]

5-Azacytidine is a pyrimidine analog of cytidine which was synthesized by Pískala and Šorm in 1964 (1). The drug acts as an analog of cytidine, interfering with the synthesis of DNA, RNA, and proteins. The mechanism of action has been investigated by a number of investigators, an extensive bibliography of which is included in a recent review by Carter and Slavik (2).

The drug exhibits experimental antitumor activity against L1210 leukemia in mice, lymphoid leukemia in AK mice, and Ehrlich ascites tumor in mice (3).[14] In a phase I study by Weiss et al (4) 30 patients with a variety of solid tumors were treated, with objective responses being reported in seven of 11 patients with carcinoma of the breast, two of five with malignant melanoma, and two of six with carcinoma of the colon. McCredie et al[15] noted a response in one patient with a renal carcinoma and in another with malignant melanoma.

In August 1972, the Southwest Oncology Group (SWOG) initiated a phase II study (SWOG-7208) in

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[3]This study was conducted by the Southwest Oncology Group (SWOG).

[4]5-Azacytidine: NSC-102816; CAS reg. No. 320-67-2; 5-triazin-2(1*H*)-one, 4-amino-1-β-D-ribofuranosyl-.

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[13]The following institutions also participated in this study: University of Kansas, Brooke Army Medical Center, Tulane University, Cleveland Clinic, Baylor College of Medicine, Ohio State University, Northwestern University, University of Oklahoma, Scott and White Clinic, Wilford Hall Medical Center, Louisiana State University, University of Virginia, Tumor Institute of Seattle, and University of Arizona.

**Reprint requests to:* SWOG, Operations Office, 3500 Rainbow Blvd, Suite 201, Kansas City, Kan 66103.

[14]Broder LE, and Carter SK. Clinical brochure: 5-azacytidine (NSC-102816). Prepared by the NCI, Bethesda, Md, 1970, 25 pp.

[15]McCredie KB, et al. A phase I study of 5-azacytidine. M. D. Anderson Hospital (unpublished data).

order to determine the effectiveness of 5-azacytidine in patients with solid tumors.

METHODS AND MATERIALS

Patients with histologically proven malignant solid tumors with measurable disease who were not eligible for higher priority protocols or more conventional chemotherapy were eligible for this study. Other criteria for eligibility included the following: written consent by the patient who had been informed as to the investigational nature of the study; a life expectancy > 8 weeks; no prior therapy with 5-azacytidine; an adequate bone marrow reserve with an absolute granulocyte count > 2000/μl and a platelet count > 100,000/μl; adequate renal and hepatic function with a BUN < 25 mg/100 ml or serum creatinine < 2 mg/100 ml and total serum bilirubin < 2 mg/100 ml unless caused by the tumor; and no chemotherapy given in the previous 21 days. Pretreatment studies included a cbc count, blood chemistries, and chest and other roentgenograms as clinically indicated. Blood counts were obtained weekly, while chemistries and roentgenograms were obtained every 3 weeks.

The initial dose of 5-azacytidine was 225 mg/m^2 by rapid iv push or infusion over 15–30 minutes on Days 1–5 every 3 weeks as tolerated. Because of myelosuppression, the dose was subsequently reduced to 175 and later to 150 mg/m^2. Subsequent courses were repeated at 3-week intervals if the nadir of the wbc and platelet counts had been reached and evidence for recovery was present. The doses in subsequent courses were adjusted according to the prior nadirs.

Two courses of treatment associated with some myelosuppression were considered necessary for an adequate trial.

The patient and his performance status, as well as the above studies, were evaluated at a minimum of every 3 weeks. Hematologic toxicity was graded according to severity.

TABLE 1.—5-Azacytidine—phase II study of solid tumors (SWOG-7208)

	No. of patients
Eligible	191
Evaluable	121
Partially evaluable	46
Early death	32
Refused further therapy	6
Lost to followup	8
Not evaluable	24
Insufficient data	16
Other	8

The response to therapy was classified as: complete regression, partial regression, stable disease, and increasing disease. Standard criteria were used with a partial regression indicating a reduction in tumor by > 50%.

RESULTS

Tumor Response

One hundred and ninety-one eligible patients from 21 institutions were entered in this study (table 1). All patients had received prior therapy. The tumor types and the response to therapy are listed in table 2.

There were five partial regressions noted for a response rate of 2.6% of the 191 eligible patients and 3% of the 167 evaluable patients. Stable disease was noted in 16 patients for a response rate of 8.4% of the eligible patients and 9.6% of the evaluable patients.

Partial regressions were noted in two patients with adenocarcinomas of the lung, one with a squamous cell carcinoma of the lung, and two with embryonal carcinomas of the testicle. The duration of the responses varied from 28 to 77 days. Three of the five patients received 225 mg/m^2 while two received 175 mg/m^2. Stable disease was noted in five of 17 patients with adenocarcinomas of the kidney, one of 14 with adenocarcinoma of the breast, two of 15 with adenocarcinomas of the colon, two of six with adenocarcinomas of the pancreas, and in single patients with malignancies arising from six other primary sites. Responses were noted at all three dose levels with the duration of the stability varying from 39 to 255 days.

Toxicity

The major toxicities associated with 5-azacytidine therapy were myelosuppressive and gastrointestinal. At the higher dose leukopenia with a wbc count < 4000/μl was noted in 83% of the patients, with severe leukopenia (a wbc count < 2000/μl) being noted in 64%. At the lower doses 63% of the patients had a wbc count < 4000/μl while in 27% it was < 2000/μl.

At the higher dose 29% of the patients developed thrombocytopenia with 8% having a platelet count < 50,000/μl. At the lower doses 12% of the patients had thrombocytopenia which was severe in 4%.

The gastrointestinal toxicity did not appear to be dose related because at all dose levels > 80% of the patients had nausea and vomiting and/or diarrhea which was severe in 33%. The nausea and vomiting was usually most severe on the first and second

TABLE 2.—Tumor types and response to therapy

Tumor	No. of evaluable patients	No. of patients with— Partial regression	Stable disease	Increasing disease
Pancreas				
Adenocarcinoma	6		2	4
Islet cell carcinoma	1		1	
Lung				
Adenocarcinoma	12	2	1	9
Squamous cell carcinoma	14	1		13
Undifferentiated cell carcinoma	12			12
Colorectal	15		2	13
Kidney	17		5	12
Breast	14		1	13
Testicle				
Embryonal cell carcinoma	2	2		
Teratocarcinoma	1			1
Choriocarcinoma	1			1
Urinary bladder	2		1	1
Primary liver carcinoma	4		1	3
Palate, adenocarcinoma	1		1	
Parotid, adenocarcinoma	3		1	2
Uterus, adenocarcinoma	1			1
Cervix, squamous cell carcinoma	4			4
Ovary	5			5
Non-Hodgkin's lymphoma	4			4
Sarcoma	7			7
Melanoma	13			13
Tongue, squamous cell carcinoma	2			2
Nasal antrum	1			1
Esophagus, squamous cell carcinoma	3			3
Stomach, adenocarcinoma	7			7
Skin, squamous cell carcinoma	1			1
Larynx, squamous cell carcinoma	1			1
Gallbladder	1			1
Unknown primary				
Adenocarcinoma	8			8
Undifferentiated cell carcinoma	4			4
Totals	167	5	16	146

days but in some patients it persisted through the entire 5-day period of administration or longer. Antiemetics, even when given in large doses, were not noticeably effective. Diarrhea often occurred 2–3 hours following drug administration and usually remitted spontaneously. Twenty of the 191 eligible patients (11%) refused further therapy mainly because of the severe gastrointestinal toxicity. Hepatotoxicity was not observed (5).

In this study there were 13 deaths attributable to drug toxicity. Eleven were due to sepsis associated with severe neutropenia while two were due to cerebral hemorrhage associated with severe thrombocytopenia. Eleven deaths occurred on the higher dose schedule (225 mg/m^2) while two occurred at the lower doses (175 and 150 mg/m^2). Eight deaths occurred during the first course of therapy with death occurring in 11 of the 13 patients within 36 days. All patients had had previous treatment with radiation therapy, chemotherapy, or both, or had bone marrow infiltration with tumor rendering them more susceptible to the myelosuppressive toxicity.

DISCUSSION

Despite the early encouraging results of the study reported by Weiss et al and the Central Oncology Group (4), the results of this study were quite disappointing. 5-Azacytidine induced a few favorable responses which were usually of poor quality and short duration. The therapy was associated with significant toxicity with a death rate of 6% of all patients entered and with 11% of the eligible patients refusing further therapy. Moertel et al (6) had a similar experience in a phase II study of 5-azacytidine in the treatment of patients with advanced gastrointestinal cancer, reporting only one transient partial response out of 29 patients treated. They also reported significant toxicity. On the other hand, it should be noted that encouraging favorable

responses to this drug have been reported in the treatment of patients with acute leukemia, both myeloblastic and lymphoblastic (7–9).

Further clinical trials may be indicated in certain solid tumor types such as embryonal carcinoma of the testicle and adenocarcinomas of the lung and kidney. Caution is advised in patients with inadequate bone marrow reserves.

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Phase II Study of 5-Azacytidine in Solid Tumors [1,2,3]

Arthur J. Weiss,* Gerald E. Metter, Thomas F. Nealon, John P. Keanan, Guillermo Ramirez, A. Swaiminathan, William S. Fletcher, Scot E. Moss, and R. W. Manthei [4,5]

SUMMARY

One hundred and seventy-seven patients were evaluated after therapy with 5-azacytidine using a dose of 1.6 mg/kg/day × 10 days followed by a maintenance regimen. One hundred and forty-eight of the patients received the drug by rapid iv infusion and 29 received the drug daily by an infusion that lasted between 18 and 24 hours. Hematologic toxicity was significant but transient in both groups. Nausea was severe using the rapid iv infusion and minimal with the slow infusion. Antitumor effect was seen in 17% of the evaluable patients with carcinoma of the breast and 21% of the patients with malignant lymphomas. Occasional responses were seen with a variety of other solid tumors. The responses were transient, and clinically, resistance to 5-azacytidine appeared to develop quite rapidly. No clear-cut difference was seen in response rates or duration of response between the two groups of patients. Cross resistance to other anticancer agents was not noted. It is believed that the drug is only of minimal value as a single agent in solid tumors using either of the methods of administration described.

[Cancer Treat Rep 61:55–58, 1977]

5-Azacytidine, a pyrimidine antagonist, was originally studied in Czechoslovakia and has received limited trials in solid tumors in the US (1–4). A phase I study by the Central Oncology Group (COG) showed some activity against breast cancer (5). The drug is known to be of value in the treatment of acute myelogenous leukemia (6) and has shown activity in patients with choriocarcinoma and hydatidiform moles (6). A phase II study was recently completed by the COG and the results of this study are presented in this paper.

MATERIAL AND METHODS

Only patients that had a histologically proven diagnosis of carcinoma with at least one clearly measurable lesion were eligible for the study. Each patient had to be between 15 and 80 years of age and could have received no prior X-ray or anticancer drug therapy in the preceding 4 weeks. The hemoglobin had to be ≥ 10 g, the wbc count ≥ 4500/mm^3, and the platelet count ≥ 125,000/mm^3. The BUN had to be ≤ 25 mg/dl and the bilirubin ≤ 1.5 mg/dl. Hematologic studies were obtained weekly during therapy and at least every 2 weeks thereafter. A BUN, bilirubin, SGOT, and alkaline phosphatase were obtained every 4 weeks. The outline of the treatment regimen is presented in figure 1.

All lesions were measured at least every 14 days. All patients were followed for at least 4 weeks after development of toxicity provided that progression of disease was evident at that time. Patients who

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[2]Supported by Public Health Service grants CA-12294 and CA-12271 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]5-Azacytidine: NSC-102816; CAS reg. No. 320-67-2; *s*-triazin-2(1*H*)-one, 4-amino-1-β-D-ribofuranosyl-. Supplied by the Cancer Therapy Evaluation Program, Division of Cancer Treatment, NCI.

[4]This study was conducted by the Central Oncology Group (COG-7123). The following institutions participated in the study: Thomas Jefferson University, Philadelphia, Pa (*Drs. Weiss and Manthei*); University of Wisconsin, Madison (*Drs. Metter, Ramirez, and Moss*); Saint Vincent's Hospital, New York, NY (*Dr. Nealon*); Cooperative Chemotherapy Program, Honolulu, Hawaii (*Dr. Keanan*); New Jersey College of Medicine, Newark (*Dr. Swaiminathan*); and University of Oregon, Portland (*Dr. Fletcher*).

[5]We gratefully acknowledge the expert technical assistance of Tana Pischer and Mary K. Hamielec.

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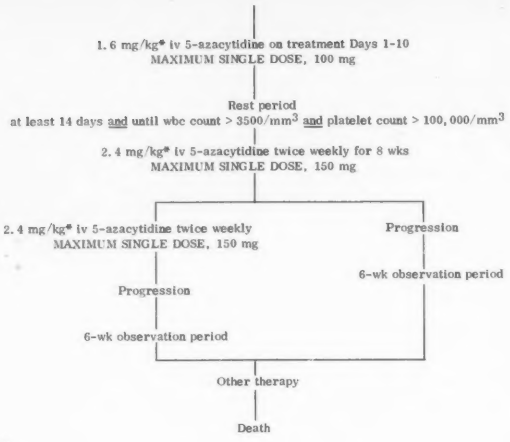
1. 6 mg/kg* iv 5-azacytidine on treatment Days 1-10
MAXIMUM SINGLE DOSE, 100 mg
Rest period
at least 14 days and until wbc count > 3500/mm3 and platelet count > 100, 000/mm3
2. 4 mg/kg* iv 5-azacytidine twice weekly for 8 wks
MAXIMUM SINGLE DOSE, 150 mg
2. 4 mg/kg* iv 5-azacytidine twice weekly
MAXIMUM SINGLE DOSE, 150 mg
Progression
Progression
6-wk observation period
6-wk observation period
Other therapy
Death

*Based on the lesser of actual and ideal body weights.

FIGURE 1.—Treatment regimen.

showed evidence of antitumor effect were followed on maintenance therapy until progression of disease occurred. To qualify as having an objective remission, the product of the two diameters of measured lesions must have decreased by ≥ 50% for a minimum period of 2 weeks.

Because of the severe nausea seen when 5-azacytidine was given by rapid iv infusion, we treated 29 patients with a protocol that was identical except that the 5-azacytidine was given over an 18–24-hour period. The drug was dissolved in Ringer's lactate solution, pH 6.7–6.8. 5-Azacytidine is stable under these conditions for 18–24 hours as shown by the following experiment. ^{14}C-labeled 5-azacytidine was added to the infusion in selected patients chosen for a pharmacologic study. Aliquots were removed from the infusion bottle at intervals during and at the completion of the infusion and the aliquots were separated by paper chromatography.[6] In all samples, 80%–100% of the radioactivity migrated as a spike with added cold 5-azacytidine.

RESULTS AND DISCUSSION

Of the 221 patients entered in the protocol, 44 were unevaluable for a variety of reasons, most of which related to protocol violations early in the course of drug therapy. The data to be presented relate to the 177 evaluable patients. Table 1 shows the toxicity seen in this study. The degree of leukopenia and thrombocytopenia was greater when the drug was given by the slow 24-hour infusion than when it was given by iv push. Hematologic suppression was transient using both methods of therapy; the interval between the nadir of leukopenia and the return of the wbc count to normal was ≤ 4 weeks in all patients treated. Nausea and vomiting commonly was severe when the drug was given by rapid iv infusion. Antiemetics commonly were of very little benefit. The nausea and vomiting was the major cause of patient resistance to further administration of the drug. When the drug was given by slow iv infusion, the nausea was either minimal or nonexistent. Stomatitis was seen in 25% of the patients receiving the drug by slow iv infusion; it was rare (7% of the patients) when the drug was given by iv push. An erythematous rash was seen in two patients receiving the drug by infusion. Hepatic failure[7] has been reported as a complication of

[6]The following systems were used for chromatography: (*a*) isobutyric acid:water:NH_4OH, 50:30:1; (*b*) N butanol saturated with water; (*c*) N butanol:water:acetic acid, 10:3:1; (*d*) NH_4AC in H_2O 7.7% adjusted to pH 7.5:95% ethanol, 3:7; and (*e*) $(NH_4)_2SO_4$ 60% in 0.1 Na_3PO_4 (pH 6.8):N propanol, 50:1.

[7]Hepatic failure was defined by the statistical office as being a rise in bilirubin to > 50% above the top normal of the reporting laboratory due clinically to drug therapy.

TABLE 1.—Summary of toxicity

	% of patients with degree* of severity of—									
	0		1		2		3		4	
Toxic effect	Iv push	Infusion	Iv push	Infusion	Iv push	Infusion	Iv push	Infusion	Iv push	Infusion
Nausea and vomiting	14	90	27	7	33	3	24	0	2	0
Diarrhea	78	97	11	3	9	0	2	0	0	0
Leukopenia	30	0	22	28	26	52	18	16	4	3
Thrombocytopenia	71	3	9	21	8	41	9	28	3	7

*Nausea and vomiting: 0 = none; 1 = nausea, no vomiting; 2 = occasional vomiting controlled by oral antiemetics; 3 = frequent vomiting, parenteral and oral antiemetics required; 4 = severe vomiting requiring parenteral antiemetics. Diarrhea: 0 = none; 1 = 1–3 loose stools daily; 2 = 3–5 loose stools daily; 3 = > 5 loose stools daily; 4 = severe diarrhea, parenteral fluids required. Leukopenia (cells/mm³): 0 = > 4000; 1 = 3000–4000; 2 = 2000–2999; 3 = 1000–1999; 4 = < 1000. Thrombocytopenia (platelets/mm³): 0 = > 125,000; 1 = 100,000–125,000; 2 = 75,000–99,000; 3 = 50,000–74,000; 4 = < 50,000.

TABLE 2.—Antitumor effects of 5-azacytidine (No. of patients with response/No. of evaluable patients)*

Tumor	Response†					
	Partial		No change		Progression	
	Rapid iv push	Infusion	Rapid iv push	Infusion	Rapid iv push	Infusion
Lung	1/22 (5%)	0/2	12/22 (55%)	1/2	9/22 (41%)	1/2
Breast	4/23 (17%)	2/6 (33%)	9/23 (39%)	1/6 (17%)	10/23 (43%)	3/6 (50%)
Large intestine	0/24 (0)	0/2	15/24 (62%)	0/2	9/24 (38%)	2/2
Melanoma	0/12		3/12 (25%)		9/12 (75%)	
Hodgkin's disease		1/6 (17%)		2/6 (33%)		3/6 (50%)
Non-Hodgkin's lymphoma		2/8 (25%)		5/8 (63%)		1/8 (12%)
Miscellaneous	9/59 (15%)		34/59 (58%)		16/59 (27%)	

*8 patients receiving drug by rapid iv push were not evaluable for response due to severe toxicity.
†Mean duration of remission (all patients) was 5 weeks (rapid iv push) and 4.5 weeks (infusion).

therapy with 5-azacytidine (7,8). This was seen in 7% of the patients receiving the drug by iv push. No patient receiving the drug by slow iv infusion developed this complication. Since almost all the patients that received their drug by iv push required antiemetic therapy, it was difficult to define the cause of the hepatic failure in these patients.

The antitumor effect of 5-azacytidine is presented in table 2. Seventeen percent of the patients with breast cancer showed evidence of an objective remission and three of 14 patients with malignant lymphomas, all of whom received the drug by slow iv infusion, developed evidence of an objective response. In all patients remissions were seen primarily with nonvisceral disease and the remissions commonly were transient and of very little value to the patient. In those patients who developed an objective response, the mean duration of remission was 5 weeks. None of the remissions could be maintained by continuous therapy with 5-azacytidine and it appeared that resistance to the agent developed rapidly. Remissions were seen in individual cases of carcinoma of the stomach, kidney, and lung, and a sarcoma. Again, these remissions were transient and are of little overall benefit to the patient. The data were analyzed to see if there was any relationship between the level of toxicity and the remission rate. There was no relationship between the degree of leukopenia and the remission rate. Significant thrombocytopenia was more common in patients that developed objective remissions. Patients that had hepatic involvement by tumor had a significantly higher remission rate (24%) than those that did not (5%), although no patient that had evidence of hepatic failure developed an objective remission. These data are presented in table 3. This difference is statistically significant especially when it is noted that in this series the most common tumor with hepatic metastases was carcinoma of the large intestine, a tumor that is resistant to 5-azacytidine as used here. While we know of no definite evidence that the liver actively degrades 5-azacytidine, the increased leukopenia and thrombocytopenia noted during therapy in the patients with hepatic metastatic disease suggests the possibility that impaired drug degradation is responsible for the increase in remission rates seen. Since the patients with hepatic failure tolerated therapy well, the data are suggestive that a higher dose may be associated with a higher remission rate.

Remissions were seen in patients whose tumor appeared to be resistant to 5-fluorouracil, cytosine arabinoside, methotrexate, and alkylating agents. Thus, clinically there appears to be no cross resistance to these agents.

While the agent as used today has only limited effect against solid tumors, it is known to be an active antitumor antimetabolite that shows essentially no cross resistance with other antipyrimidines. For that reason, explorations of other methods of administration as a single agent, or in combination, are believed to be worthy of consideration.

TABLE 3.—Hepatic involvement*

Group	Response						Drug stopped† (No. of patients)	Drug death† (No. of patients)	Total No. of patients
	Partial		No change		Progression				
	No. of patients	%	No. of patients		No. of patients	%			
No liver metastasis or failure	6‡	6	58§	56	40¶	38	6	2	112
Liver metastasis without liver failure	8	31	11‡	42	7	27			26
Liver failure	0	0	4	40	6	60			10

*The data were analyzed using both the Kruskal-Wallish test and chi-square analysis: H = 7.91, $P = 0.19$.
†Not used in the calculation of response rate.
‡1 patient also had subjective progression.
§3 patients also had subjective progression and in 1 patient the drug was stopped due to toxicity.
¶1 patient also had subjective no change and in 1 patient the drug was stopped due to toxicity.

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Combination Chemotherapy With Cyclophosphamide, Vincristine, Methyl-CCNU, and Bleomycin in Advanced Bronchogenic Carcinoma: Experience With 106 Patients [1,2,3]

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SUMMARY

A phase II study was conducted with three different combinations of cyclophosphamide, Oncovin (vincristine), methyl-CCNU, and bleomycin (COMB) in 106 patients with advanced bronchogenic carcinoma of all histologies. The response rates were 27.3% for COMB I (six of 22 patients), 18.2% for COMB II (eight of 44 patients), and 7.5% for vincristine, methyl-CCNU, and bleomycin (three of 40 patients). Complete responses were seen only in the small cell carcinoma group. Duration of remission and overall survival were of significance for the small cell group, whereas survival for the other histologic types comparing responders to nonresponders was of no statistical significance. Toxicity was significant and included nausea and vomiting, neuropathy, and myelosuppression. These drug combinations were disappointing and only in small cell carcinoma was meaningful antitumor activity seen.

[Cancer Treat Rep 61:59–64, 1977]

The chemotherapy of advanced bronchogenic carcinoma has persistently remained ineffective despite worthwhile advances in therapy with other solid tumors. Single-agent chemotherapy, the most commonly used mode of treatment, has consistently produced a low level of response rate without meaningful prolongation of survival. Combination chemotherapy, having made dramatic inroads in improving response rate and survival in Hodgkin's and non-Hodgkin's lymphoma, breast carcinoma, as well as soft-tissue sarcoma, seemed a logical approach to the therapy of advanced bronchogenic carcinoma.

Methyl-CCNU has shown superiority to other nitrosoureas in the Lewis lung cancer system (1). In a phase II study conducted by the Southwest Oncology Group (2), methyl-CCNU achieved an overall response rate of 12.5% in 160 evaluable patients with disseminated bronchogenic carcinoma. Cyclophosphamide, the most widely used alkylating agent employed for solid tumors, has a reported overall response rate of 23% in bronchogenic neoplasms (3). The combination of methyl-CCNU and cyclophosphamide was found to be highly synergistic against Lewis lung carcinoma (1).

Vincristine, marginally effective in bronchogenic carcinoma, causes an accumulation of cells in M phase that is maximum at 6–12 hours after administration (4). Since bleomycin has maximal cytotoxic effect in M phase, it seemed reasonable to combine these two drugs taking advantage of the stathmokinetic effect of vincristine and the relative cycle specificity of bleomycin (5,6). Indeed, while bleomycin alone had a response rate of 6.8% in extensive

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[2]Supported by Public Health Service grants CA-07127 and CA-08096 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

Bleomycin: NSC-125066; CAS reg. No. 11056-06-7; [2,4'-bithiazole]-4-carboxylic acid, 2'-(2-aminoethyl)-, monohydrate.

All drugs were supplied by Developmental Therapeutics Program, Division of Cancer Treatment, NCI.

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lung carcinoma, the combination of vincristine and bleomycin (given 6 hours later) resulted in a response rate of 25% (7). Additionally, these two drugs have no significant myelosuppression and are attractive for inclusion in combination-chemotherapy regimens. Initial clinical experience combining these four drugs (cyclophosphamide, Oncovin [vincristine], methyl-CCNU, and bleomycin [COMB]) was particularly encouraging with respect to advanced bronchogenic carcinoma (7).

Based on the above observations, a phase II study was designed to answer the following questions: (*a*) What is the antitumor activity of COMB and Oncovin (vincristine), methyl-CCNU, and bleomycin (OMB)? (*b*) Is twice weekly vincristine and bleomycin therapy more effective than weekly administration given over a more protracted period of time? (*c*) Are methyl-CCNU and cyclophosphamide synergistic in human tumors? (*d*) Is full-dose methyl-CCNU as effective as the methyl-CCNU and cyclophosphamide combination? (*e*) Can significant prolongation of survival time be achieved in patients considered to have responded to therapy?

MATERIALS AND METHODS

In order to be eligible for entrance in this study patients must have satisfied the following criteria: (*a*) histologic confirmation of carcinoma of the lung with evidence of incurability by conventional means; (*b*) evidence of metastatic spread beyond the limits of one radiation portal; (*c*) measurable disease outside of the radiation field; and (*d*) no prior exposure to any of the four drugs under investigation. Patients were randomized by the closed-envelope technique between the three treatment regimens outlined in table 1. Randomization was deliberately weighted in favor of the COMB II and OMB treatment arms since we had accrued sufficient data in an earlier phase II study with the COMB I schedule.

In the COMB I schedule, vincristine and bleomycin were given twice weekly for 12 weeks with a total of 300 units of bleomycin administered. The COMB II schedule consisted of weekly vincristine and bleomycin over a 24-week period with a total of 300 units of bleomycin administered. The dosage and scheduling of cyclophosphamide and methyl-CCNU in the COMB I and COMB II arms were identical. The OMB arm consisted of full doses of methyl-CCNU, the deletion of cyclophosphamide, and the identical doses of vincristine and bleomycin as given in the COMB I arm.

Bleomycin was discontinued if pulmonary toxicity developed, withheld if mucositis and/or dermatologic toxicity was manifest, and restarted at the time of full clinical recovery. Vincristine was permanently discontinued if evidence of severe neuropathy developed, ie, footdrop, obstipation, abdominal pain, or the inability to write, button clothes, or use food implements.

Methyl-CCNU and cyclophosphamide dosages were reduced 25% for subsequent courses of therapy if there was significant leukopenia (< 2000 cells/mm^3) and/or thrombocytopenia (< 75,000 platelets/mm^3). Subsequent courses of therapy were delayed until there was sufficient evidence of recovery from the nadir granulocyte and platelet counts observed with the previous course of therapy.

Patients were evaluated serially while receiving

TABLE 1.—Treatment scheme

Combination	Drug	Schedule and route of administration
COMB I	Cyclophosphamide	1000 mg/m^2 iv, Day 1 and q6wks
	Methyl-CCNU	100 mg/m^2 orally, Day 1 and q6wks
	Vincristine	0.75 mg iv, Day 2 and twice weekly × 6 wks, then 0.5 mg iv twice weekly × 6 wks
	Bleomycin	15 mg im, Day 2 and twice weekly × 6 wks, then 10 mg im twice weekly × 6 wks (6 hrs after vincristine)
COMB II	Cyclophosphamide	1000 mg/m^2 iv, Day 1 and q6wks
	Methyl-CCNU	100 mg/m^2 orally, Day 1 and q6wks
	Vincristine	0.75 mg iv, Day 2 and weekly × 12 wks, then 0.5 mg iv weekly × 12 wks
	Bleomycin	15 mg im, Day 2 and weekly × 12 wks, then 10 mg im weekly × 12 wks (6 hrs after vincristine)
OMB	Methyl-CCNU	200 mg/m^2 orally, q6wks
	Vincristine	0.75 mg iv, Day 2 and twice weekly × 6 wks, then 0.5 mg iv twice weekly × 6 wks
	Bleomycin	15 mg im, Day 2 and twice weekly × 6 wks, then 10 mg im twice weekly × 6 wks (6 hrs after vincristine)

therapy with respect to cbcs, pulmonary function tests, chest roentgenograms, liver and renal function tests, and tumor measurements.

Complete remission was defined as the disappearance of all clinical evidence of active tumor for a minimum of 4 weeks together with a disappearance of all tumor-related symptoms. Partial remission was defined as $> 50\%$ decrease in the sum of the products of the largest perpendicular diameters of all measured lesions lasting at least 4 weeks in the absence of significant increase in any one lesion or the appearance of new lesions. Progressive disease was defined as unequivocal increase of any measured lesion or the appearance of new lesions. Patients were considered as having received an "adequate trial" if they received at least 2 weeks of therapy and were defined as having an "early death" if death occurred within the first 14 days of therapy. All reported responses occurred outside concurrently treated radiation therapy portals.

Demographic Data

A total of 106 patients were entered in the study. Twenty-two patients were randomized to the COMB I treatment arm, 44 to the COMB II arm, and 40 to the OMB arm. The smaller number of patients in the COMB I arm is accounted for by the fact that a large phase II study had been conducted with COMB I prior to the initiation of this study, thus between the two studies a sufficient number of patients had been accrued on this therapy so that additional numbers of patients were not needed.

Table 2 outlines the demographic data of the patients on each arm. With respect to histology, sex, race, prior radiotherapy, concurrent radiotherapy, prior chemotherapy, prior surgery, age, and performance status there was no statistically significant difference between the three arms. Mean disease-free interval was significantly longer ($P < 0.05$) for the OMB patients when compared to the COMB II, and is accounted for by the greater number of patients on COMB II with no disease-free interval ($P < 0.05$).

There were seven early deaths on COMB I and COMB II and eight on OMB, all attributed to infectious complications or organ failure. One patient on COMB II and one on OMB therapy were lost to followup.

With regard to documented major sites of meta-

TABLE 2.—Demographic data

	Combination		
	COMB I	COMB II	OMB
Histology			
Squamous cell carcinoma	9 (40%)	22 (50%)	25 (62%)
Small cell carcinoma	7 (32%)	10 (23%)	7 (17%)
Large cell carcinoma	3 (14%)	6 (14%)	3 (8%)
Adenocarcinoma (including alveolar and bronchiolar)	3 (14%)	4 (9%)	4 (10%)
Other (not classified)	—	2 (4%)	1 (3%)
Totals	22	44	40
Sex			
Men	16 (73%)	29 (66%)	28 (70%)
Women	6 (22%)	15 (34%)	12 (30%)
Race			
Caucasian	16 (73%)	34 (77%)	26 (65%)
Prior radiotherapy	9 (40%)	17 (39%)	16 (40%)
Concurrent radiotherapy	5 (23%)	6 (14%)	6 (15%)
Prior chemotherapy	1 (5%)	3 (7%)	1 (3%)
Prior surgery	21 (95%)	36 (82%)	37 (92%)
Mean disease-free interval (wks)	5	9	16
No. of patients with disease-free interval	16 (59%)	33 (75%)	21 (53%)
Median age (yrs)	56.5	55.3	58.5
Median performance status (Karnofsky)	69.2	65.8	66.8

TABLE 3.—Response rate, median duration of remission (wks), and survival (wks)

Histology	Combination								
	COMB I			COMB II			OMB		
	No. of responders/No. treated	Duration of remission (range)	Survival (range)	No. of responders/No. treated	Duration of remission (range)	Survival (range)	No. of responders/No. treated	Duration of remission (range)	Survival (range)
Squamous cell carcinoma									
Responders	1/9	10	30.0	2/21	12(8–16)	16.7(13.4–20)	2/24	6.3*	35.1*
Nonresponders			3.85(0.1–47.8)			9.1(0.6–61.7)			9.0(1.3–58.6)
Adenocarcinoma									
Responders	1/3	2	8.1	1/4	15	23.4	0/4		
Nonresponders			8.35(5.7–11.0)			4.85(3.7–6.0)			48(9.3–48.3)
Small cell carcinoma									
Responders	4/7	13.75(4.6–52)	35.3(16–71)	4/10	15.35(8.3–26.9)	23.1(23–33.9)	1/7	14.7	20.1
Nonresponders			15.1(5.4–31.1)			12.7(0.3–20.9)			12.6(5.3–23.6)
Large cell carcinoma									
Responders	0/3			0/6			0/3		
Nonresponders			22.7(0.9–46)			22.6(6.9–54.3)			8.3(6–11.6)
Other									
Responders				1/2	6.7	11.7	0/1		
Nonresponders						8			0.7

*1 patient lost to followup.

static disease (lung, lymph nodes, liver, bone, bone marrow, and brain) there was no statistical difference between the three treatment arms.

RESULTS

Response and Survival

The overall response rates (including early deaths) for the three arms of the study are as follows: COMB I, 27.3% (six of 22 patients); COMB II, 18.2% (eight of 44); and OMB, 7.5% (three of 40). Table 3 enumerates response rate by histology and specific therapy as well as median duration of remission and duration of survival of responding and nonresponding patients. There was no statistically significant difference for response rates between the three arms of the study. Four complete remissions were seen and were all in the small cell carcinoma subtype: two in the COMB I arm and one each in the COMB II and OMB arms. In the small cell group there appeared to be significant prolongation of median survival in the responding group, however because of the small number of patients there was statistical confirmation in only the COMB II group ($P = 0.03$).

Toxicity

Myelosuppression was almost universally seen. Cumulative toxicity was not seen nor was there any evidence that subsequent courses of chemotherapy had to be delayed to allow for hematologic recovery in patients receiving concurrent radiation therapy. Both COMB I ($P < 0.025$) and COMB II ($P < 0.05$) had a significantly higher incidence of leukopenia (< 2000 cells/mm^3) than OMB. However, OMB patients who received full doses of methyl-CCNU had a higher incidence of thrombocytopenia (< 100,000 platelets/mm^3) than COMB I ($P = 0.2$) or COMB II ($P = 0.0003$).

Nausea and vomiting was consistently seen in all patients treated. There was no difference seen in any of the three treatments. The incidence of neuropathy was highest in the COMB I-treated patients as opposed to a prior report in which a more protracted course of vincristine resulted in a higher incidence of neurologic complications (8). Pulmonary toxicity, confirmed clinically, radiographically, or histologically, was not reported in COMB I-treated patients but was seen in COMB II-treated patients (2.3%) and OMB patients (7.5%). Alopecia occurred more frequently in COMB I (22.7%) and COMB II (29.5%) as opposed to OMB (7.5%) and was attributed to cyclophosphamide. As previously reported (8), a "debilitation syndrome" was seen in all three treatment regimens. There were no deaths attributed to drug-related causes.

DISCUSSION

Despite encouraging early reports from both laboratory and clinical studies, our results in advanced bronchogenic carcinoma utilizing three different schedules of combination chemotherapy with COMB were disappointing. Not only were the overall response rates low, but meaningful prolongation of survival was not achieved. A direct comparison with Livingston's series (8) is not possible, as detailed information on the population treated in his series is not available. Factors such as extent of disease, performance status, prior therapy, and immunologic status all may have a bearing when the two different population groups are compared.

Significant improvement in median survival for oat cell carcinoma when it is compared to placebo or single-agent chemotherapy is obvious (9). However, COMB offers no particular advantage over other reported chemotherapy combinations (10) and the more striking results seen recently in combination radiotherapy and chemotherapy regimens (10–12).

In summary, combination chemotherapy as detailed in this report offered no new insights or inroads into the therapy of advanced bronchogenic carcinoma. Response rates and ultimate survival of patients were poor, at the expense of drug toxicity. Small cell carcinoma continues to be the most responsive tumor type and the one in which significant prolongation of survival can be achieved with combination chemotherapy.

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Pilot Study of Two Adriamycin-Based Regimens in Patients With Advanced Malignant Lymphomas [1,2,3]

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SUMMARY

Forty-two patients with advanced malignant lymphomas, all of whom had failed at least one prior course of chemotherapy, were treated with one of two new combination chemotherapy regimens to determine patient tolerability. Twenty-seven patients received regimen 1, after which this study was discontinued, and subsequently 15 additional patients were treated with regimen 2. Regimen 1 (BAP) consisted of BCNU and adriamycin administered iv on Day 1 and prednisone administered orally on Days 1–5 with cycles repeated at 21-day intervals. Regimen 2 (VAP) consisted of VP-16-213 given iv on Days 1 and 2, adriamycin given iv on Day 3, and prednisone given on Days 3–7 in a schedule designed to produce cell cycle synchronization; cycles were repeated at 21–28-day intervals. Both regimens were, in general, well tolerated. Reversible bone marrow depression was the major toxic reaction observed. Nine of 27 patients treated with regimen 1 and five of 15 patients treated with regimen 2 experienced objective tumor regressions. Tolerable dosage levels of both regimens have been defined for future clinical trials.

[Cancer Treat Rep 61:65–68, 1977]

Although most patients with malignant lymphomas will initially respond to currently available chemotherapy, the majority will ultimately relapse, reflecting only temporary suppression of the neoplastic process. Therefore, there exists a need for the development of non-cross-resistant systemic therapy for patients with malignant lymphomas who become refractory to conventional approaches. This feasibility study was designed to study two new combination chemotherapy programs in patients with refractory malignant lymphomas, with the primary aim to determine patient tolerability and define acceptable dosage levels for future comparative clinical trials.

Regimen 1 (BCNU, adriamycin, prednisone; BAP) was developed on the basis of the known single-agent activity of BCNU (1,2) and adriamycin (3) in patients with malignant lymphomas.[5] Prednisone, a nonmyelosuppressive agent, was added because of its direct antineoplastic effect (4,5) as well as its palliative effects on the systemic symptoms which frequently are a major source of morbidity in these patients. It was elected to employ a relatively low dose of BCNU in order to allow more frequent drug administration without incurring severe bone marrow depression.

Regimen 2 (VP-16-213, adriamycin, prednisone; VAP) also was developed on the basis of the single-agent activity of its constituents.[6] VP-16-213 has been shown to have activity in patients with advanced lymphomas (6–9). The dosage schedule selected was based on the report of encouraging antitumor activity observed in a series of patients with refractory malignant lymphomas treated with vincristine and cyclophosphamide on a schedule de-

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[3]Adriamycin: NSC-123127; CAS reg. No. 23214-92-8. Drugs used in this investigation were provided by the Eastern Cooperative Oncology Group.

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[5]BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-.

[6]VP-16-213: NSC-141540; 4'-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside).

signed to produce tumor cell synchronization (10). We elected to utilize VP-16-213 as the synchronizing agent for two reasons: (*a*) most of our treatment failures have previously received vincristine and are refractory to its effect, and (*b*) epipodophyllotoxin derivatives have been shown to be able to produce cell cycle synchronization in vitro (11). We employed adriamycin rather than cyclophosphamide on Day 3 as the cytostatic agent because most of our patients had received extensive alkylating agent therapy prior to the study.

MATERIALS AND METHODS

All patients entered into these studies had histologic proof of Hodgkin's disease or non-Hodgkin's malignant lymphoma. Seventy-eight percent and 93% of the patients on regimens 1 and 2 respectively had received prior radiation. All patients had failed at least one trial of chemotherapy and were not considered appropriate candidates for further radiation therapy because of widespread disease. Two patients had received prior adriamycin therapy. Each patient had one or more evaluable indicator lesions to assess response to therapy. Pretreatment wbc counts were $\geq$ 4000 cells/mm^3 except for two patients on regimen 1 (wbc counts, 2300 and 3900 cells/mm^3) and two patients on regimen 2 (wbc counts, 2000 and 3500 cells/mm^3). Pretreatment platelet counts were > 100,000 cells/mm^3 except in one patient on regimen 1 (97,000 platelets/mm^3) and one patient on regimen 2 (81,000 platelets/mm^3). Renal function was adequate as indicated by a serum creatinine level of < 1.5 mg/100 ml or a serum urea level of < 50 mg/100 ml. Total bilirubin was < 1.5 mg/100 ml.

The following factors excluded patients from the study: radiation therapy or chemotherapy within the preceding 3 weeks, active cardiac disease as defined by angina pectoris, myocardial infarction within the past 3 months, clinical features of congestive heart failure or persistent electrocardiographic abnormalities, a moribund status with expected survival of < 1 month, or underlying medical problems such as uncontrolled diabetes mellitus or peptic ulcer which might be aggravated by steroid administration.

BAP Regimen

The BAP regimen is outlined in table 1. BCNU and adriamycin were given iv on Day 1 and prednisone was given orally on Days 1–5. Starting doses were as follows: BCNU (30 mg/m^2), adriamycin (15 mg/m^2), and prednisone (100 mg/m^2). The prednisone dose was kept constant in subsequent cycles, although adriamycin and BCNU doses were escalated in some patients in whom no myelosuppression was observed. After initial experience with this regimen was gained, the starting doses of adriamycin and BCNU for patients subsequently entered into this study were escalated incrementally to a maximum starting dose of 60 mg/m^2 for both adriamycin and BCNU. BCNU and adriamycin doses were decreased on subsequent cycles if hematologic recovery (wbc count > 4000 cells/mm^3 and platelet count > 100,000 cells/mm^3) was not evident on Day 21.

Pretreatment patient characteristics are outlined in table 2.

VAP Regimen

The VAP regimen consisted of VP-16-213 administered by slow iv infusion over 20–30 minutes on Days 1 and 2, adriamycin given by iv injection on Day 3, and prednisone given orally on Days 3–7 (table 1). Treatment was repeated at 21–28-day intervals until the patient showed signs of disease progression. The dose of VP-16-213 was kept constant at 80 mg/m^2 and the prednisone dose was also fixed at 100 mg/m^2. Patients with extensive prior radiation therapy and chemotherapy received adriamycin at a dose of 30 mg/m^2 on their first cycle of

TABLE 1.—Drug administration schedules

Regimen	Treatment days						
	1	2	3	4	5	6	7
BAP							
BCNU	×						
Adriamycin	×						
Prednisone	×	×	×	×	×		
VAP							
VP-16-213	×	×					
Adriamycin			×				
Prednisone			×	×	×	×	×

TABLE 2.—Pretreatment patient characteristics

Drug regimen	Age (yrs)		Sex		Histology									
					Non-Hodgkin's lymphomas									
					Histiocytic			Mixed			Lymphocytic			
	Range	Median	Male	Female	Nodular	Diffuse	Not known	Nodular	Diffuse	Not known	Nodular	Diffuse	Not known	Hodgkin's disease
BAP	17–75	50	18	9	0	6	3	2	3	1	1	5	0	6
VAP	20–69	54	8	7	1	4	1	2	0	1	2	0	0	4

therapy. The remaining patients received adriamycin at a dose level of 45 mg/m^2. The dose of adriamycin was adjusted up or down on subsequent cycles depending upon the severity of hematologic toxicity and degree of recovery of the wbc and platelet counts prior to the next course of therapy.

Pretreatment patient characteristics are summarized in table 2.

RESULTS

BAP Regimen

Mild to moderate gastrointestinal upset, satisfactorily controlled with phenothiazines, was noted in approximately one third of the patients. Several patients developed alopecia, and one patient experienced fever which may have been drug related. Another patient experienced an episode of gastrointestinal bleeding following the third cycle of therapy which may have been induced or aggravated by prednisone administration.

Hematologic toxicity from the BAP regimen is summarized in table 3. All dose levels which were given for five or more cycles are included for analysis. Nadir and Day-21 wbc and platelet counts are indicated as well as the number of cycles for which these data were available. Hematologic toxicity consisted primarily of leukopenia which in general had resolved by Day 21. Cumulative toxicity was not a prominent feature of this regimen, since only two of the 14 patients receiving four or more cycles of therapy required downward modifications of adriamycin and BCNU due to leukopenia. One patient died of progressive lymphoma and sepsis following the first course of therapy. The preterminal wbc count was 2000 cells/mm^3 and the platelet count was 200,000 cells/mm^3.

Nine of 27 patients treated experienced objective tumor regressions. None of the seven nonambulatory patients treated with this regimen experienced tumor regression.

VAP Regimen

Mild nausea and occasional vomiting, related primarily to adriamycin administration, was observed in several patients. Alopecia commonly developed. A single patient developed local urticaria following adriamycin administration. A second patient developed a reversible encephalopathy which was attributed to steroid administration. One episode of sepsis was observed associated with severe leukopenia and was successfully treated with antibiotics.

Hematologic toxicity from the VAP regimen is summarized in table 3. Two patients treated with adriamycin at the 30-mg/m^2 dose level experienced severe leukopenia (wbc nadirs, 300 and 400 cells/mm^3 respectively). Both patients had received extensive prior radiation therapy, one patient had known bone marrow involvement by lymphoma, and both patients had marked elevations of serum alkaline phosphatase and SGOT. Four patients treated at the 45-mg/m^2 adriamycin dose level experienced a wbc nadir < 1000 cells/mm^3. All of these patients had received extensive prior radiation therapy, one was leukopenic prior to therapy (wbc count, 2000 cells/mm^3), and two had significant abnormalities of serum alkaline phosphatase and SGOT. Two of these four patients, however, had normal pretreatment liver function tests and wbc values. There was no apparent evidence of cumulative toxicity, although only four patients received four or more cycles of therapy. Significant thrombocytopenia (platelet count, $< 50{,}000$ cells/mm^3) was observed in three patients. All had received extensive prior radiation therapy and had significant abnormalities of serum alkaline phosphatase and SGOT, and one patient was thrombocytopenic (platelet count, 81,000 cells/mm^3) prior to treatment. Recovery of bone marrow suppression was evident by Day 28 in all evaluable treatment cycles.

Five of 15 patients treated experienced objective tumor regression. Only one of the six nonambulatory patients treated with the VAP regimen experienced a tumor regression.

TABLE 3.—Hematologic toxicity

Drug dosage (mg/m²)		Cycles administered	Wbc counts (cells × 10^{-3}/mm³) Nadir			Wbc counts Day 21†			Platelet counts (platelets × 10^{-3}/mm³) Nadir			Platelet counts Day 21†		
			Range	Median	N*	Range	Median	N	Range	Median	N	Range	Median	N
Adriamycin	*BCNU*					*Regimen 1—BAP*								
15	30	34	1.4–21.5	4.7	12	2.1–47.2	6.0	27	45–386	159	8	105–769	365	27
20	40	5	3.8–6.6	4.9	3	4.2–7.2	5.3	5	253–348	276	3	240–322	265	5
30	40	7	—	—	0	4.7–8.3	6.0	6	—	—	0	184–543	480	6
30	60	27	2.9–5.9	4.3	4	2.3–14.6	4.5	21	251–339	287	4	222–561	311	20
60	60	10	1.3–10.2	1.8	7	2.0–5.5	3.6	8	78–540	125	7	129–539	198	8
Adriamycin	*VP–16–213*					*Regimen 2—VAP*								
22.5	80	4	3.7–5.0	4.4	4	4.1–4.6	4.2	3	149–311	268	4	263–276	270	3
30	80	15	0.3–11.6	3.6	8	3.5–9.8	6.8	10	35–550	120	7	126–655	351	10
45	80	18	0.3–11.2	2.7	15	3.4–13.9	5.9	11	8–613	178	15	145–842	303	11
50	80	4	4.0–4.1	—	2	3.5–8.4	4.7	3	—	—	1	112–861	175	3

*N = No. of cycles following which nadir or Day-21 (or Day-28 for VAP) counts were available.
†Day 21 or 28 for VAP regimen.

DISCUSSION

Neither the BAP nor the VAP regimen was associated with unacceptable acute reactions. The relatively modest doses of BCNU and adriamycin employed in these studies resulted in only mild to moderate gastrointestinal upset which was usually well controlled with phenothiazines. Severe bone marrow depression was observed only in patients receiving the VAP regimen. Abnormalities of liver function appeared to have been a contributing factor, which is consistent with the known importance of biliary excretion for the disposition of both VP-16-213 and adriamycin.

On the basis of data accumulated in this study, we recommend the following cytotoxic drug dosages for future clinical trials: (*a*) *BAP regimen*—adriamycin (60 mg/m²) and BCNU (60 mg/m²) every 21 days in previously untreated patients with normal liver function. In patients with extensive prior radiation, chemotherapy, or abnormalities of liver function (SGOT or alkaline phosphatase), we recommend 30 mg/m² of adriamycin and 60 mg/m² of BCNU. (*b*) *VAP regimen*—VP-16-213 (80 mg/m² on Days 1 and 2 recommended as a cell cycle-synchronizing dose). In previously untreated patients with normal liver function, adriamycin at a dose of 45 mg/m² on Day 3 is suggested. An initial adriamycin dose of 15–30 mg/m² is recommended for patients with extensive prior radiation, chemotherapy, or significant abnormalities of serum alkaline phosphatase or SGOT.

Antitumor activity of both regimens has been demonstrated in patients with advanced refractory malignant lymphomas. However, no comparison of the relative efficacies of these regimens is possible from the nonrandomized, sequential studies presented in this report. It is of interest to note that 13 of the 29 ambulatory patients (45%) but only one of the 13 nonambulatory patients (8%) experienced objective tumor regression, confirming the importance of performance status as a prognostic sign in this patient population.

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5-Fluorouracil in Childhood Solid Tumors [1,2,3]

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Brief Reports and Preliminary Communications

Few clinical trials with 5-fluorouracil (5-FU) have been conducted in children (1–3). The Pediatric Division of the Southwest Oncology Group designed a study to determine the effectiveness of 5-FU, given twice weekly, against solid tumors of children. Studies in adults have shown that daily 5-FU is exceedingly toxic (4,5) and that a weekly dose is as effective but less toxic (6). It was reasoned, therefore, that a twice-weekly schedule might be preferable.

METHODS

Seventy-eight children with advanced metastatic disease and measurable lesions were given 5-FU in doses of 400 or 600 mg/m^2 of body surface area. Although there are conflicting reports concerning the relative efficiency of 5-FU administered orally compared with the iv route (7,8), the drug was administered iv unless such administration was not feasible, in which case it was given orally.

RESULTS

Table 1 summarizes the results of treatment of these 78 children. Because of advancing disease, there were 11 early deaths, six < 9 days after initiation of therapy and five between 11 and 18 days after therapy. 5-FU was discontinued in three instances because of toxicity: severe vomiting after two doses of 5-FU in one child and neutropenia in two children. There were no complete responses. Seven partial responses occurred and will be described. In an eighth child, a scalp lesion, due to metastatic neuroblastoma, improved while additional lesions appeared elsewhere.

CASE REPORTS

Patient No. 1

Five months after a mid-thigh amputation for osteogenic sarcoma, a 14-year-old boy had a lesion 2 cm in diameter in the left lung and a lesion 13 mm in diameter in the right lung. No response was achieved with daunorubicin; in 4 weeks a pleural effusion filled 50% of the left hemithorax. After ten doses (400 mg/m^2 iv) of 5-FU, the pleural effusion markedly decreased and the lesion in the right lung disappeared. After 500 rads of irradiation to both lung fields, the lesion in the left lung disappeared. 5-FU was continued for 14 months. Three years after cessation of 5-FU the child remains free of any evidence of disease.

Patient No. 2

An 11-year-old girl had pulmonary metastases 11 months after an above-the-knee amputation for osteogenic sarcoma. After 10 mg of mechlorethamine intrapleurally at the time of diagnosis and eight doses (600 mg/m^2 orally) of 5-FU given twice weekly, the pulmonary lesion decreased from 35 × 35 mm to 12 × 15 mm and remained so for 128 days. Because of vomiting, 5-FU was given as a single weekly dose after the first 4 weeks of treatment.

Patient No. 3

Tumor cells in the bone marrow of a child with disseminated neuroblastoma cleared after five doses (600 mg/m^2 iv) of 5-FU but recurred when the marrow was examined 30 days later.

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[3]5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8.

[4]This study was conducted by the Southwest Oncology Group. The following members participated in the study: *M. E. Haggard* (University of Texas Medical Branch, Galveston); *A. Cangir* (University of Texas M. D. Anderson Hospital, Houston); *A. H. Ragab* (Emory University School of Medicine, Atlanta, Ga); *D. Komp* (University of Virginia School of Medicine, Charlottesville); *J. Falletta* (Duke University Medical Center, Durham, NC); and *G. B. Humphrey* (University of Oklahoma Medical Center, Oklahoma City).

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TABLE 1.—Distribution of solid tumors treated at 2 dose levels with a twice-weekly schedule

Disease	No. of patients	No. of patients treated ≤ 3 wks with—		No. of patients treated ≥ 4 wks with—		
		Early death	Progressive disease	Progressive disease	Responses	
					None	Partial
		400-mg/m² dose				
Neuroblastoma	8	1	4	2		
Rhabdomyosarcoma	6	3	1	2		
Osteogenic sarcoma	4		1	2		1
Ewing's sarcoma	6		1	2	1	1
Miscellaneous	3	1		1	1	
Total	27	5	7	9	2	2
		600-mg/m² dose				
Neuroblastoma	8	3		2	2	1
Rhabdomyosarcoma	6	1	1	3		
Osteogenic sarcoma	8		3	1	3	1
Ewing's sarcoma	6		3	1	1	1
Teratoma, malignant	4		1	2	1	
Wilms' tumor	4		1	3		
Retinoblastoma	3		1	2		
Hepatic tumors	5	1		3	1	
Miscellaneous	7	1	1	1	1	2
Total	51	6	11	18	9	5

Patient No. 4

A 14½-year-old girl with Ewing's sarcoma and rapidly progressing metastatic lung lesions experienced a > 50% decrease in pulmonary nodules after six doses (600 mg/m² orally) of 5-FU, but died unexpectedly 5 days later.

Patient No. 5

A 9-year-old boy with Ewing's sarcoma demonstrated a 50% decrease in size of four metastatic pulmonary lesions after 6 weeks of therapy (400 mg/m² iv) but progression of lesions began 6 weeks later.

Patient No. 6

A child with stage IV Hodgkin's disease, previously sensitive to a number of agents, had resolution of hepatomegaly and pleural effusions with disappearance of fever, night sweats, and pruritis after five doses of 5-FU (600 mg/m² iv). Disease recurred after 100 days.

Patient No. 7

An 11-year-old girl with an undifferentiated neoplasm, which was initially observed radiographically as a mass 10 cm in diameter extending above the right hemidiaphragm and involving the chest wall, had failed to respond to actinomycin D, vincristine, and cyclophosphamide. After four doses of 5-FU, there was subjective improvement and an ultimate decrease in size of the mass to 5.8 cm after 20 doses (400 mg/m² iv) of 5-FU. The tumor remained stable during weekly therapy for 5 months and then began to increase in size.

TOXICITY

Four of 15 patients who received six or more doses of 400 mg/m² of 5-FU had leukocyte levels of < 2000/mm³ and three had platelet levels of < 100,000/mm³. Nine of 29 patients receiving three or more doses of 600 mg/m² of 5-FU had leukocyte levels of < 2000/mm³ and four had platelet counts of < 100,000/mm³. Nausea and vomiting and diarrhea were observed but required cessation and/or reduction of therapy in only ten patients. Mucositis was severe in only one patient. Toxicity was equally frequent in children receiving 5-FU iv and those receiving it orally.

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Mode of Decrease of Leukemic Cells and Leukocytes During Neocarzinostatin Treatment [1,2]

Brief Reports and Preliminary Communications

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In order to find the correlation of the decrease of leukemic cells in the blood to that in the bone marrow or to that of leukocytes in response to antileukemia drugs, we have been investigating the decrease curves of cells during the treatment of acute leukemia for several years. Recently, neocarzinostatin (NCS), discovered by Ishida, was found effective against acute leukemia (1). In the phase II study of NCS, the decrease curves of leukemic cells and neutrophil leukocytes were analyzed in the manner which has been previously reported (2,3). The information obtained from this analysis seems to be useful for the comparison of antileukemia drugs and for the determination of response and duration of response to NCS as a predictive system.

MATERIALS AND METHODS

Eighteen patients with acute leukemia were treated with 2 mg/day (adults) or 1 mg/day (children) of NCS for 5–18 days, until the decrease of leukemic cells slowed down or ceased. During these treatment courses, decrease curves of leukemic cells in the blood and bone marrow and of leukocytes were analyzed as previously reported. Leukocyte differential was measured by counting 200–5000 leukocytes as previously described. For the measurement of leukemic cells in the bone marrow, the bone marrow was aspirated five to ten times during the decrease. Nucleated cell counts were plotted on semilogarithmic paper and adjusted using a French to a smooth curve. The product of adjusted cell count and the percentage of leukemic cells were used for the computation. The parameters obtained were compared with those obtained in patients treated with 20 mg/kg/day of cyclocytidine (CC) or 40 mg of daunorubicin (DM) for 4 days.[4]

RESULTS AND DISCUSSION

Remission Rate

In 18 patients with acute leukemia treated with NCS, one complete (5.5%) and five partial (27.7%) remissions were produced. The parameters of these remissions are shown in table 1. There were statistically significant differences in the maximum rate of decrease, number of leukemic cells at nadir, and log decrease compared to those patients without remission.

Decrease of Leukemic Cells in Blood

As shown in table 1, statistically significant differences between NCS and CC or DM were observed in the maximum rate and duration of decrease, the number of leukemic cells at nadir, and the log decrease. No significant difference was observed in the number of leukemic cells before treatment. NCS depletes leukemic cells in the blood more rapidly than CC, less rapidly than DM, and for a shorter period of time than CC.

The log decrease of NCS was significantly lower than with DM. This parameter seems to indicate the direct effectiveness of this drug. It is not modified by infection or bleeding and can be compared with other drugs. The low log decrease in the blood with NCS would be one of the reasons why the remission rate of NCS alone is not satisfactorily high.

Decrease of Neutrophil Leukocytes

The maximum rate of decrease and the log decrease of neutrophil leukocytes were lower than

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[2]Neocarzinostatin: NSC-157365; CAS reg. No. 4507-13-5.,

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[4]Cyclocytidine: NSC-145668; CAS reg. No. 10212-25-6; 6*H*-furo[2',3':4,5]oxazolo[3,2-*a*]pyrimidine-2-methanol, 2,3,3*a*,9*a*-tetrahydro-3-hydroxy-6-imino-, monohydrochloride.

Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6.

TABLE 1.—Parameters in the decrease of leukemic cells in blood and neutrophil leukocytes*

Drug	No. of patients	Parameters: Maximum rate of decrease (log/day)	Rate of deceleration (log/day/day)	Time lag to maximum decrease (day)	Duration of decrease (day)	No. of cells in total blood (log): Before treatment	At nadir	Decrease
			Decrease of leukemic cells					
NCS	18	−0.3892 ±0.1735	0.07690 ±0.09520	2.9 ±2.21	9.8 ±2.67	9.80 ±0.92	8.43 ±1.05	1.37 ±0.69
NCS, patients with remission	6	−0.5291† ±0.2042	0.05681 ±0.19390	2.8 ±2.55	10.1 ±2.68	9.19 ±0.93	7.29† ±0.56	1.90† ±0.61
CC	14	−0.2634‡ ±0.1109	0.02764 ±0.02518	3.4 ±3.06	17.0‡ ±6.35	8.97 ±2.93	7.15‡ ±0.81	1.75 ±0.55
DM	11	−0.5979‡ ±0.1559	0.10450 ±0.07572	3.4 ±1.14	11.3 ±4.37	10.08 ±1.26	7.46 ±1.47	2.64‡ ±1.10
			Decrease of neutrophil leukocytes					
NCS	15	−0.2103§ ±0.0839	0.04766 ±0.04488	4.1 ±2.43	11.0 ±4.48	9.85 ±0.67	9.06 ±0.65	0.89§ ±0.31
CC	14	−0.1804 ±0.1584	0.02275 ±0.02460	4.4 ±3.06	14.3 ±5.18	9.41 ±0.58	8.53§ ±0.83	0.88§ ±0.72
DM	11	−0.3662‡,§ ±0.1743	0.06744 ±0.04599	4.9 ±3.33	10.6 ±3.71	9.78 ±0.98	8.43 ±1.03	1.35‡,§ ±0.67

*Mean ± standard deviation
†Statistically significant difference from nonremission patients in NCS group ($P < 0.05$).
‡Statistically significant difference from NCS group.
§Statistically significant difference from leukemic cells in the same drug group.

TABLE 2.—Parameters in the decrease of leukemic cells in bone marrow*

Drug	No. of patients	Decrease of leukemic cells: Maximum rate of decrease (log/day)	Rate of deceleration (log/day/day)	Time lag to maximum decrease (day)	Duration of decrease (day)	No. of leukemic cells/μl bone marrow (log): Before treatment	At nadir	Decrease
NCS	6	−0.1909 ±0.1218	0.04083 ±0.03629	5.7 ±1.09	13.4 ±2.74	4.96 ±0.44	3.81 ±0.66	1.15 ±0.41
CC	6	−0.4345† ±0.1394	0.05233 ±0.01780	2.3† ±1.98	11.5 ±3.47	4.77 ±0.50	3.05 ±0.59	1.72 ±0.49
DM	9	−0.4348† ±0.2171	0.1025 ±0.1046	3.7† ±1.69	9.4† ±3.44	5.03 ±0.50	3.51 ±0.77	1.52 ±0.58

*Mean ± standard deviation.
†Statistically significant difference from NCS group.

those of leukemic cells in the same drug group (table 1). A significant difference in the maximum rate of decrease and the log decrease of neutrophil leukocytes was also shown between NCS and DM. The injury to neutrophil leukocytes by NCS seems milder than that caused by DM.

Decrease of Leukemic Cells in Bone Marrow

Statistically significant differences between NCS and CC or DM were observed in the maximum rate of decrease, the time lag to the maximum decrease, and the duration of decrease. NCS started to deplete leukemic cells in the bone marrow later with a lower maximum rate of decrease than CC or DM although NCS decreased the leukemic cells in the blood more rapidly than CC. The action of NCS on the leukemic cells in the bone marrow seems milder than those of CC or DM (table 2).

From the parameters that have been mentioned it seems that NCS may be used in those instances where a rapid decrease of leukemic cells in the

blood, but not an extremely low level of leukocytes, is preferred, as reported in a case of monocytic leukemia with a high leukemic cell count and complicated by infection (2).

Because NCS is not very powerful, as shown by the low log decrease, it seems likely that combination therapy would prove better than a single drug administration. The parameters discussed seem to be useful for comparing antileukemia drugs. It may be helpful for an antileukemia drug evaluation to estimate those parameters at an early stage of a phase II study.

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Chemotherapy of Alveolar Soft Part Sarcoma: A Case Report [1,2]

Brief Reports and Preliminary Communications

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Alveolar soft part sarcoma is a rare soft tissue tumor arising in skeletal muscle. The uncertain histogenesis of the tumor and its unusual biologic behavior has fascinated pathologists and clinicians for decades. The indolent course of the disease, the almost universal tendency for late widespread metastases to occur, and its predilection for teenagers and young adults who are otherwise healthy, make the treatment of this tumor a particular challenge. While surgical excision of the primary tumor seems an acceptable way to approach the patient initially, metastases are frequently not prevented by surgery alone and there is as yet no reliable means for treating them when they become manifest.

We report a case of alveolar soft part sarcoma that has shown tumor sensitivity to two chemotherapeutic agents: adriamycin and intermediate-dose methotrexate with citrovorum factor rescue.[5] We suggest that a reliably effective antitumor chemotherapy regimen may eventually be an integral part of the early management of all patients with alveolar soft part sarcoma.

CASE REPORT

On December 5, 1973, a 19-year-old man underwent excision of a 5 × 9-cm tumor in the belly of the left soleus muscle. Pathologic examination by light and electron microscopy disclosed an alveolar soft part sarcoma. Bilateral multiple pulmonary nodules were found by whole-chest tomography, and in January 1974 the patient received cycloleucine[6] therapy. The tumors, initially stable, grew slowly and after 10 months the cycloleucine was discontinued. In November 1974 iv adriamycin (75 mg/m^2) was started on an every-3-week basis. This therapy was continued for 9 months with dosage modifications and continual monitoring of his cardiac status which remained normal. By August 1975 a diminution in size of most of the pulmonary nodules had occurred; several were no longer apparent by roentgenography and no new lesions appeared during this partial remission.

Because the patient had received 790 mg/m^2 of adriamycin at this time, and because this tumor characteristically pursues an indolent course, thoracotomy was performed on August 21, 1975, to resect the larger tumor nodules. At operation the left lung was found to contain myriad small tumors that were not discernible by roentgenographic examination. Ten small nodules were removed from the left lower lobe and confirmed pathologically to be alveolar soft part sarcoma. The following day the patient received methotrexate (240 mg/m^2) with citrovorum rescue 42 hours later (25 mg every 8 hours for 48 hours). Thereafter, 0.5–1.0 g/m^2 of methotrexate was given by iv push every 3 weeks with the same citrovorum rescue on an outpatient basis. There was only one episode of serious toxicity which was treated without hospitalization. The patient developed moderately severe stomatitis, a generalized morbilliform rash, and severe leukopenia and thrombocytopenia. After recovery, a 2-day water diuresis and alkalinization of the urine was undertaken with each course of therapy and the methotrexate was continued. On November 24, 1975, repeat tomography disclosed a reduction from 21 to 16 mm and 22 to 14 mm of the diameters of the largest pulmonary nodules when compared to identical tomographic cuts before methotrexate. Therapy was continued, but in December 1975 the serum transaminases were found to be in the 300–500-units/ml

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[5] Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.
Adriamycin: NSC-123127; CAS reg. No. 23214-92-8.
Citrovorum factor: NSC-3590; CAS reg. No. 6055-86-5; glutamic acid, *N*-[*p*-[[(2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]-, calcium salt.

[6] Cycloleucine: NSC-1026; CAS reg. No. 52-52-8.

range. Percutaneous liver biopsy disclosed minimal fatty change and some increased cellularity in the peri-portal spaces but no fibrosis or disruption of the hepatic architecture. Mild methotrexate hepatotoxicity was diagnosed. Since the pulmonary nodules continued to regress, no new lesions appeared, and the transaminases returned to normal within a week, therapy was continued. The methotrexate-induced remission lasted 9 months, at which time several small new pulmonary nodules were noted. The patient then received vincristine, actinomycin D, and cyclophosphamide in combination with no objective decrease in the lesions. At the time of writing he is asymptomatic, but slight progression has been noted after 3 months of the latter regimen.

DISCUSSION

Over 80 cases of alveolar soft part sarcoma have been reported in the medical literature (3). The tumor arises in striated muscle, usually in the extremities, and has a peculiar 2 to 1 preference for the right side of the body (3). In the series studied by Lieberman et al (5), seven of 53 patients had metastases when first seen; of the 36 with apparently localized disease at presentation who were followed, 20 developed metastatic disease by the time the patients were reported. Local recurrence was unusual. The sites of metastases were pulmonary in 42%, bony in 19%, and cerebral in 15%, indicating a hematogenous mode of spread.

The tendency for late metastases was noted in the early reports. This tendency often leads to an erroneous impression of cure after local excision. The median survival, however, was 5.6 years in Lieberman's entire group of 50 patients. The very few patients < 20 years of age had a much better prognosis with a median survival of 19.0 years; most of the patients were · 20 years, and in these cases the median survival was 4.1 years. After reviewing his information Lieberman concluded "our material indicates that we are dealing with an often indolent, but always fatal disease."

Under the microscope the tumor appears as clusters of polygonal cells giving an organoid or pseudoglandular appearance (2,7). The cells are grouped into "alveoli" of ten to 50 cells. There are many non-argyrophilic cytoplasmic granules which lend a granular, refractile quality to the cytoplasm. There is often an incomplete basement membrane. The tumor has variously been called non-chromaffin paraganglioma, granular cell myoblastoma, and alveolar soft part sarcoma.

The ultrastructure of the tumor (9,10) is characterized by abundant rough and smooth endoplasmic reticulum, large numbers of mitochondria, and a well-developed Golgi apparatus that is often associated with electron-dense, membrane-bound granules. A characteristic feature noted by several observers is the occasional presence of large rhomboid intramicrosomal crystals which have a filamentous structure when examined by higher magnifications. Theories of histogenesis of this singular tumor fall into two groups. Marshall et al (6) and Fisher et al (4) feel that the tissue is somehow of myogenous origin, while Smetana et al (7) proposed in 1951 that the tumor is of neural derivation, closely related to paragangliomas and chemodectomas. Welsh et al (10), after extensive study of the ultrastructure, came to the latter conclusion and recently Unni et al (9) agreed. One of the difficulties with this view has been the inability of anatomists to find paraganglia in skeletal musculature. Whatever the cell of origin, pathologists have agreed to use the descriptive term alveolar soft part sarcoma.

In view of the inevitable distant lesions that develop in these patients, it is safe to conclude that nearly all patients have micrometastases at the time of presentation. One method of approach, then, would be an effective chemotherapeutic exposure at the time of diagnosis, based on the rationale that has made adjuvant chemotherapy successful in patients with osteogenic sarcoma, Wilms' tumor, embryonal rhabdomyosarcoma, and now perhaps mammary carcinoma.

There is little in the literature of this rare tumor to provide a background for chemotherapy. Talley (8) reported tumor regression of two undifferentiated sarcomas in 1972 but it seems unlikely that either of these tumors were alveolar soft part sarcoma. Hoeg et al (1) collected a small series of patients and described chemotherapy in two patients. The first derived no benefit from prednisone, anabolic steroids, cyclophosphamide, or thioTEPA. The second patient had resolution of pulmonary metastases with thioTEPA. The remission lasted 2½ years, at which time a local recurrence was successfully treated with the same drug. Our patient represents another modest chemotherapeutic success. Both adriamycin and intermediate-dose methotrexate induced partial remissions, albeit of short duration. It would seem reasonable that if one or more reliably active agents can be identified for this disease most patients should receive postoperative chemotherapy for a finite period of time in the hope of eliminating the insidious microscopic foci of tumor.

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Phase II Study of Dianhydrogalactitol and ICRF-159 in Patients With Advanced Breast Cancer Previously Exposed to Cytotoxic Chemotherapy [1,2,3]

Brief Reports and Preliminary Communications

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Since multiple-drug programs have come into use, the objective response rate to chemotherapeutic agents in patients with advanced breast cancer has risen to 50%–60% and the duration of responses has at least doubled compared to previous single-drug use (1). Previous experience, however, has clearly shown the necessity to develop new effective drug programs to be utilized in the event of primary multiple-drug failure. The search for effective new agents led to this study utilizing two agents available for assessment.

The study assessed two new agents, dianhydrogalactitol and ICRF-159, in a randomized clinical trial in a group of patients having previously failed cytotoxic chemotherapy. Dianhydrogalactitol was discovered to be the major conversion-reaction product of dibromodulcitol upon treatment with mild alkali or human serum.[5] Dibromodulcitol has been regarded as an alkylating agent on the basis of its chemical activity and cross resistance to alkylating drugs (2). A major activity of the halogenated hexitols is thought to be related to the conversion of the diepoxide galactitol (3). Dianhydrogalactitol as a daily infusion on each of 5 successive days was assessed at our institution in a phase I study. The major significant and dose-limiting toxicity was myelosuppression (4).

ICRF-159 was developed at the Imperial Cancer Research Fund facilities in London and was selected for clinical trials on the basis of antitumor activity in L1210 leukemia and the Lewis lung tumor model. Schedule dependency was noted, with the intermittent schedule being superior to daily administration. ICRF-159 inhibits DNA synthesis and blocks progression of cells through the G_2-M phase of the cell cycle.[6] Again, myelosuppression seemed to be the most predictable and the dose-limiting toxicity associated with ICRF-159 therapy (5).

MATERIALS AND METHODS

Thirty-two patients with advanced breast cancer entered this randomized trial. All of the patients had previously been exposed to cytotoxic chemotherapy and 20 of the 32 patients had been exposed to two or more multiple-drug programs. Ten of the 32 patients had had previous exposure to at least one multiple-drug program. Only two of the 32 patients had been previously exposed to single-drug therapy. Twenty-seven of the 32 patients had visceral-dominant disease while the other five had soft tissue-dominant disease. All patients were postmenopausal. In all cases, serum creatinine and serum bilirubin levels were < 2 mg/dl. All of the patients had measurable disease and an expected survival of at least 2 months with a performance score (Eastern Cooperative Oncology Group) of three or better. Pre- and post-treatment assessments were as previously described (1). The definition of an objective clinical response was standard: a $\geq 50\%$ decrease in the product of the perpendicular diameters of all measurable disease without evidence of new lesions or an increase of $> 25\%$ of previous lesions.

Dianhydrogalactitol was administered by rapid iv infusion on each of 5 successive days at a dose of 30 mg/m^2 in 13 of the 24 patients who received it as primary or secondary therapy, while ten received 25

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[2] Supported in part by contract CM-53772 from the Division of Cancer Treatment, National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3] Dianhydrogalactitol: NSC-132313; CAS reg. No. 23261-20-3; galactitol, 1,2:5,6-dianhydro-.

ICRF-159: NSC-129943; CAS reg. No. 21416-87-5; 2,6-piperazinedione, 4,4'-propylenedi-, (±)-.

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[5] Handelsman H. Clinical brochure: galactitol (NSC-132313). Prepared by the Division of Cancer Treatment, NCI.

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[6] Wasserman TH, et al. Clinical brochure: (±)1,2-di(3,5-dioxopiperazin-1-yl)propane, ICRF-159 (NSC-129943). Prepared by the Division of Cancer Treatment, NCI, revised Oct 1973.

mg/m² (one patient received 20 mg/m²). ICRF-159 was given at a dose of 750 mg/m²/day for 3 days in divided doses every 8 hours in all patients. Subsequent re-treatment, if the patients failed to progress in the interval period, occurred 4 weeks after treatment if toxicity had abated. There were no dose escalations when dianhydrogalactitol was used as secondary therapy. With ICRF-159, there was one dose escalation to 900 mg/m²/day for 3 days and one dose reduction to 400 mg/m²/day for 3 days.

RESULTS

Of the 32 patients entered in the trial, 18 received dianhydrogalactitol as primary therapy and 14 received ICRF-159. None of these patients achieved an objective clinical response as defined. Three of the 18 patients receiving dianhydrogalactitol as primary therapy did have stability of disease lasting 4, 8, and 12 weeks respectively, while four of 14 patients had stability of disease following primary therapy with ICRF-159 lasting 5, 8, and 14 weeks respectively. None of the six patients receiving dianhydrogalactitol as secondary therapy had stability of disease but four of the eight patients receiving ICRF-159 did have stability of disease, the longest of which lasted 8 weeks.

Following ICRF-159 as primary therapy, a mean survival of 11 weeks (median, 9 weeks) was achieved, with four patients continuing to survive at the time of this report, the longest of which was 34+ weeks. With dianhydrogalactitol, a mean survival of 17 weeks (median, 14 weeks) was achieved, with eight patients continuing to survive at the time of this report, the longest of which is 43 weeks.

Toxicity was as expected and included myelosuppression as the primary and dose-limiting phenomenon. Both agents produced leukopenia of a significant magnitude. Sixteen of 24 patients had wbc counts < 3000 cells/mm³ following dianhydrogalactitol and 18 of 22 patients had wbc counts < 3000 cells/mm³ following ICRF-159. ICRF-159 seemed relatively platelet sparing compared to dianhydrogalactitol with only three of 22 patients having platelet counts < 100,000 cells/mm³ while 13 of 24 patients had platelet counts < 100,000 cells/mm³ after dianhydrogalactitol. The myelosuppression following dianhydrogalactitol had a mean duration considerably longer than that following ICRF-159: 22 versus 9 days.

There were two drug-related deaths, one with each agent following severe myelosuppression and sepsis.

While some of the patients in this clinical trial did experience brief stability of their disease in the face of previous progression, neither agent was able to produce solid evidence of antitumor effect as reflected by diminution of tumor size. However, previous drug exposure to a host of multiple-drug programs including alkylating drugs may well have resulted in the inclusion of only those patients having tumors cross resistant to these agents. Phase II studies must continue in an effort to look for agents which do not manifest cross resistance in patients who fail current multiple-drug programs. The mean survival of both groups following chemotherapy was long enough to allow adequate observation of possible antitumor effect, supporting the value of assessing new chemotherapeutic agents in a group of such patients. In the future, however, perhaps drugs not found to be cross resistant to previously used compounds on animal tumor screens should be used.

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Methyl-CCNU for Patients With Previously Treated Metastatic Neuroblastoma—A Phase II Study [1,2,3]

Brief Reports and Preliminary Communications

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This study was part of a series of chemotherapy protocols for patients with neuroblastoma being conducted by the Children's Cancer Study Group (CCSG). An attempt is being made to determine the response rate of neuroblastoma to various anticancer agents so that the effective agents can be combined to form an improved regimen for newly diagnosed patients with metastatic disease. The fact that patients with neuroblastoma have not achieved improved survival following multimodal therapy, as have the patients with Wilms' tumor and soft tissue sarcoma, is the basis of the CCSG's concerted attack.

Methyl-CCNU (meCCNU) was selected for two reasons. First, it was one of a new class of compounds not previously studied in neuroblastoma, but had demonstrated antitumor activity in man; and, second, the late nadir of bone marrow toxicity, ie, 30–40 days, suggested it might be combined with other agents having an earlier period of depression, without additive toxicity.

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[2]Supported by Public Health Service grant CA-13539 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

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MATERIALS AND METHODS

Patient Selection

To be eligible for the study, patients had to have metastatic neuroblastoma with at least one measurable lesion, had to be resistant to conventional agents, and must have had no previous therapy with nitrosourea compounds. Each patient entered was expected to be alive for at least 2 months. The hematologic status required a total neutrophil count of ≥ 1500 cells/mm^3 and a platelet count of ≥ 75,000 platelets/mm^3 unless there was concurrent tumor infiltration of the bone marrow. Since most patients had already taken part in other CCSG studies of neuroblastoma, central review of the histology was not required.

Drug Administration

MeCCNU is one of a group of nitrosourea compounds developed at the Southern Research Institute.[11] It is the most lipid soluble of the clinically useful nitrosourea compounds which include BCNU and CCNU.[12] Its anticancer effect is thought to be produced by alkylation (1). Preliminary human pharmacology suggests that the drug is well absorbed from the gastrointestinal tract. After oral administration of radioactively labeled drug, 1%–2% of the label appears in the stool and 75% is accounted for in the urine (2). Response rates have been reported to be between 0 and 23% and responses have been seen in patients with carcinoma of the lung, lymphoma, central nervous system tumors, and melanoma (3,4).

MeCCNU was obtained from the Developmental Therapeutics Program, Division of Cancer Treatment, NCI. It was prepared in capsules and given orally at a dose of 200 mg/m^2. It was given early in the day on an empty stomach. A course consisted of

[11]A special report to the Cancer Chemotherapy National Service Center, NCI. A summary of experimental data on nitrosourea. Southern Research Institute, Dec 1969.

[12]Comis RL, et al. Clinical brochure: MeCCNU. Prepared by the NCI, 1972.

one single oral dose every 6 weeks. Local X-ray therapy was permitted to lesions other than the index lesion.

Study Parameters

During the course of treatment, wbc and platelet counts were performed weekly; changes in physical examination and measurable disease were recorded every 3 weeks. Bone marrow aspirations and radiographs to evaluate the effect of treatment were performed every 6 weeks, as were urinary catecholamines if originally elevated. Dose modifications for subsequent courses were outlined in the protocol and were dependent on the lowest total neutrophil and platelet counts; reductions were made if the neutrophil count fell to < 1000 cells/mm^3 and the platelet count to $< 100{,}000$ platelets/mm^3. In view of the tendency for cumulative toxicity with this agent, no instructions were given for increasing the dose to > 200 mg/m^2.

RESULTS

Entries and Evaluation

Thirty-four patients were entered in the study and the effect of treatment was evaluable in 23 of these. One patient was ineligible because the diagnosis was other than neuroblastoma, and ten were considered unevaluable for measurement of response since they died < 14 days from the first day of treatment. All patients had previously received cyclophosphamide and were resistant to conventional doses.

Response

Of the 23 evaluable patients, one had complete clearing of tumor from the bone marrow which lasted for 3 months. No other objective responses were seen nor was any relief of symptoms reported. Only two patients had more than one course of treatment. The median survival time of all 33 patients was 27 days (range, 2–122).

Toxicity

Toxicity was manifested mainly by thrombocytopenia. The median platelet count at the start of treatment was 106,000 platelets/mm^3 and 19 of 21 patients had tumor infiltration in the marrow (in two patients the marrow status was not recorded). In 14 of 23 patients, the platelet count fell to $< 20{,}000$ platelets/mm^3 (median 16,000 platelets/mm^3) with the nadir occurring at 28 days. No patient died from overt hemorrhage, although some died at home and autopsies were not performed on all. If the ten patients who survived 42 days or longer are analyzed, the median lowest platelet count of 23,000 platelets/mm^3 occurred at 38 days. The single patient who responded to treatment, with infiltration of the marrow, had an initial platelet count of 665,000 platelets/mm^3, showed no evidence of thrombocytopenia or neutropenia during two courses, and had his lowest platelet count of 130,000 platelets/mm^3 at the end of the three courses when the marrow became reinfiltrated with tumor. Neutropenia was neither as severe nor as frequent as thrombocytopenia; three of 23 patients developed values <500 cells/mm^3.The nadir was seen at Day 21 or at Day 41 in those patients surviving past the 42nd day. Anemia (Hgb < 8 g) occurred in 22 patients. Transient vomiting was seen in two patients, an unusually low incidence of this side effect. Since most of the children were outpatients, the actual incidence may not have been recorded correctly.

DISCUSSION

The results of treatment were disappointing. Even excluding the ten patients who did not survive for 14 days, a response rate of 4% (one of 23 patients) suggests that meCCNU is not useful in the treatment of neuroblastoma. The possibility that an inadequate drug dose was given probably can be excluded by the fact that 14 of 23 patients developed severe thrombocytopenia. A fall in platelet counts disproportionate to neutropenia is more suggestive of a drug effect rather than thrombocytopenia secondary to marrow infiltration. Other investigators have reported a similar disproportionate sensitivity of megakaryocytes to meCCNU (3). It is unfortunate that several patients were preterminal and only two received more than one course of therapy. However, neuroblastoma is a tumor sensitive to chemotherapy and the vast majority of patients who respond to other agents do so within the first few days of treatment, and certainly by 3 weeks. It is reasonable to expect that an antitumor effect, if it existed, would have occurred during the same period of time as the bone marrow depression.

All patients had received prior chemotherapy with vincristine, cyclophosphamide, and DTIC and many had received additional drugs such as adriamycin. The previous treatment with alkylating agents may have induced resistance to meCCNU and it is possible that a therapeutic effect would be seen if the drug were administered earlier in the course.

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Combination VM-26 and Adriamycin for Metastatic Transitional Cell Carcinoma [1,2,3]

Brief Reports and Preliminary Communications

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Only three drugs (adriamycin, 5-fluorouracil, and mitomycin C) have been used extensively enough in patients with metastatic transitional cell carcinoma from the urinary bladder to demonstrate relative activity in this disease, according to a review by Carter (1). Recently, an epipodophyllotoxin derivative classified as a spindle poison, VM-26, was shown by the European Organization for Research on the Treatment of Cancer to be active in bladder cancer (2). Pavone-Macaluso et al (3) have subsequently confirmed that iv VM-26 is capable of causing total regression of bladder carcinoma. The lack of significant toxicity suggested that VM-26 might be a logical drug to use in combination chemotherapy.

MATERIALS AND METHODS

Twenty-seven patients with metastatic transitional cell carcinoma from the urinary bladder were entered into this study between July 1, 1974, and June 30, 1976. Requirements for admission into the study included: (*a*) histologically confirmed transitional cell carcinoma of the urinary bladder; (*b*) a leukocyte count $> 2000/mm^3$; (*c*) a platelet count $> 100{,}000/mm^3$; and (*d*) measurable metastatic disease. The rating of symptoms and performance status was as follows: 0 = no symptoms present, patient working full-time; 1 = symptoms present, patient fully ambulatory but unable to work full-time; 2 = patient confined to bed < 50% of the time; 3 = patient confined to bed > 50% of the time; 4 = patient bedridden.

The patient status information is summarized in table 1. Objective tumor response was determined by serial measurement. The grading of response was: *complete response*—disappearance of all measurable tumor; *partial response*—a 50% decrease in the product of the maximum diameter and the perpendicular maximum diameter of all measurable disease; *stable response*—no change in any of the measurable lesions and no evidence of new lesions; and *no response*—continued growth of measurable disease or evidence of new lesions.

All patients received VM-26, 60 mg/m^2 iv on Days 1 and 2, followed by adriamycin, 50 mg/m^2 iv on Day 3. In the absence of bone marrow toxicity, the dose of adriamycin was escalated by 15 mg/m^2 in subsequent courses (for four patients). In patients with severe myelosuppression, the dose of adriamycin was decreased by 15 mg/m^2 (for two patients). The dose of adriamycin was reduced by 25% if the serum bilirubin was between 1.4 and 3.0 mg/100 ml and by 50% if it was > 3.0 mg/100 ml. The treatment program was repeated at 21-day intervals for a minimum of three courses. The total dose of adriamycin for any patient was limited to 550 mg/m^2.

RESPONSE AND TOXICITY

There were five partial responses (19%) which occurred after one to four courses of therapy (median, two courses). The mean survival of the responders was 17.8 months as opposed to 6.3 months for the nonresponders ($P < 0.01$). Two of 17 patients with visceral metastases showed partial responses lasting for 18 months (corpora and glans of the penis) and 27 months (liver), respectively. Three of nine patients with lymph node metastases achieved a partial response and, in two instances, subsequent radical cystectomy with lymphadenectomy allowed for the complete eradication of all known disease with both patients alive at 24 months. The third patient died from a probable pulmonary embolus after five courses of therapy had resulted in the complete disappearance of his supraclavicular ade-

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[3] VM-26: NSC-122819; 4'-demethylepipodophyllotoxin, 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside).

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8.

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nopathy and a marked decrease in his mediastinal adenopathy. In the one patient with abdominal wall metastases there was no response. Two patients evidenced fistula formation between the small bowel and skin, presumably as a result of tumor necrosis.

Toxic manifestations occurred in 25 patients (93%). Alopecia was recorded in 23 instances (85%), thrombocytopenia in 12 (44%), and severe leukopenia (ie, wbc count $< 1000/mm^3$) and stomatitis in two (7%).

TABLE 1.—Patient status

No of evaluable patients	27
No. of women	7
Mean age (yrs, range)	64 (56–82)
No. of patients receiving prior therapy	
Radiation (> 4000-rad tumor dose to pelvis)	9
Surgery (ileal conduit diversion)	11
Mean symptom status*	2.3

*See Materials and Methods.

DISCUSSION

The reported use of chemotherapy in patients with metastatic bladder carcinoma has been limited and has generally demonstrated the relative refractiveness of this tumor to a variety of drug programs. Our finding of only five responses (none complete) in 27 patients with metastatic transitional cell carcinoma treated with combination VM-26 and adriamycin is not significantly different from the results reported with VM-26 when used alone (3), and it is considerably less than the 36% reported response rate for adriamycin given alone (4,5). Confirmation of these reports, however, awaits the result of a randomized study now being conducted under the egis of the National Bladder Cancer Project.

Our investigation, nevertheless, suggests that if VM-26 is to be used as a synchronizing agent followed by other drugs acting in different phases of the cell cycle, its dosage may need to be increased. The current program was exceedingly well tolerated although one third of the patients had received prior extensive pelvic irradiation (> 4000 rads, tumor dose) and > 40% had undergone supravesical urinary diversion.

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Adriamycin-Cytosine Arabinoside Therapy for Adult Acute Myelocytic Leukemia [1,2,3]

Brief Reports and Preliminary Communications

Harvey D. Preisler,* Sigurdur Bjornsson, and Edward S. Henderson [4]

Recently, Yates et al (1) and Rai et al (2) reported that > 70% of patients with acute myelocytic leukemia (AML) who received therapy consisting of 7 days of cytosine arabinoside (ara-C) and daunorubicin (DNR) given on Days 1, 2, and 3 attained remissions. We report that substitution of adriamycin (ADR) for DNR in this regimen permits the administration of a lower dose of anthracycline while producing comparable rates of remission induction.

METHODS

All patients between the ages of 15 and 70 with AML were eligible for treatment with ADR, 30 mg/m²/day iv on Days 1, 2, and 3, and ara-C, 100 mg/m²/day iv on Days 1–7. Each ADR dose was administered over 20 minutes while the ara-C was given by a continuous infusion. The first 15 patients treated with this regimen were consecutive admissions; the last six patients were randomly assigned to receive this therapy as opposed to an alternate combination of ara-C and DNR.

Evaluation of therapy results was based upon criteria established by the Acute Leukemia Group B (3). A complete remission (CR) was represented by a bone marrow containing < 5% blast cells with normal cellularity and normal peripheral blood cell counts. A partial remission (PR) was similar with the exception that the bone marrow contained > 5% but < 25% blast cells. Normal bone marrow cellularity is 2+, a 0 cellular marrow is markedly devoid of cells, and a 4+ cellular marrow is packed with cells.

RESULTS

Twenty-one patients with AML or one of its variants were treated with the combination of ADR-ara-C (table 1). There were 13 responses (62%) of which 12 were complete. There were eight CRs and one PR among ten patients who were < 40 years old (table 1). Two of four patients between 41 and 60 years old and two of seven patients > 60 years achieved a CR (table 1).

Four of 14 patients < 60 years of age did not attain CR status. Three of these patients had received therapy for a prior neoplasm. One of these patients attained PR status while two died on Days 4 and 14, respectively, after the start of therapy. A necropsy of the former patient revealed persistent leukemia while a necropsy of the latter patient failed to demonstrate persistent leukemia. The fourth patient died 14 days after completing two courses of therapy without evidence of persistent leukemia.

Five of seven patients who were > 60 years old did not attain remission status and all died during the attempted remission induction. Two patients died after having received less than a complete course of therapy (Days 5 and 7 of therapy). Necroscopic examination demonstrated a persistence of leukemia in both patients. A third patient died 5 days after completing a second course of therapy. A post-mortem examination showed that her marrow was hypocellular with a few clumps of myeloblasts. The other two patients died 11 days after the end of one course of therapy and 10 days after the end of a second course of therapy, respectively; there was no evidence of leukemia.

Of the 13 responses to therapy, seven were achieved after a single course and six after two courses. Table 2 provides detailed information regarding the clinical courses of our patients. Those patients who attained remission after a single course were in remission 27.8 ± 0.6 days after the initiation of therapy (median, 28 days). Five patients who required two courses of therapy to achieve response were in remission 55 ± 5 days after the initiation of therapy (median, 52 days). The sixth patient was given the second course of treatment 8 weeks after attaining a PR.

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[3]Adriamycin: NSC-123127; CAS reg. No. 23214-92-8.

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride.

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TABLE 1.—Patient characteristics and disease status

Patient No.	Age	Sex	Leukemia type*	No. of courses of therapy	Response	Reason for response failure	Comments
				Age range, 20–39 yrs			
1	25	F	AML	2	CR		—
2	32	F	AML	1	CR		—
3	27	M	APML	2	PR		History of ALL†
4	28	F	APML	1	CR		—
5	25	M	AML	1	CR		—
6	28	M	AMMOL	½	Died	Pneumonia	History of Hodgkin's disease
7	28	M	AMMOL	1	CR		Previous therapy for AML, 2 yrs of CR
8	22	F	AML	1	CR		—
9	31	F	AML	2	CR		—
10	32	F	AML	2	CR		Previous therapy for AML, 1 yr of CR
				Age range, 40–59 yrs			
11	59	M	AML	1	Died	Mucormycosis, candidiasis	History of multiple myeloma, no AML at autopsy
12	49	F	AML	2	Died	Candidiasis, Klebsiella sepsis, aspergillosis	No AML at autopsy
13	44	F	AML	2	CR		—
14	53	F	AML	1	CR		—
				Age, ≥ 60 yrs			
15	61	M	AML	2	Died	Candidiasis, cerebral hemorrhage	No AML at autopsy
16	60	M	AML	1	Died	Gastric hemorrhage	No AML at autopsy
17	67	F	AML	2	Died	Myocardial infarction	—
18	70	M	AML	1	Died	Subdural hematoma, bronchopneumonia	—
19	62	F	AML	1	CR		—
20	64	M	AML	½	Died	Aspergillosis	—
21	66	F	AML	2	CR		—

*APML = acute promyelocytic leukemia; AMMOL = acute myelomonocytic leukemia.
†ALL = acute lymphocytic leukemia.

After a single course of therapy, the cellularity of the bone marrows of 13 patients became ≤ 1+ (all had been 3–4+ at initiation of therapy). Six of these patients attained CR after a single course of therapy, and two patients required a second course of therapy to attain CR; one additional patient received two courses of therapy and achieved a PR. Four patients required two courses of therapy to achieve marrow hypocellularity; three of these patients attained CR and one died. For four remaining patients (of the 21 treated patients) there are no data with which the cellularity of the marrow can be evaluated. Three of these patients died prior to a second marrow aspirate and one patient demonstrated a CR after the second aspirate.

The 21 patients experienced 46 episodes of fever. Of these, 14 episodes were present at the time of their admission to the hospital. An infectious cause of the febrile episode could be documented in only 18 instances. Antibiotics were administered during each febrile episode. All patients except four received granulocyte transfusions (for fever unresponsive to antibiotic therapy). Two of the patients who did not receive granulocyte transfusions attained CR and two died.

DISCUSSION

Thirteen of 21 patients with AML treated with the combination of ADR and ara-C responded to

TABLE 2.—Patient response to therapy

Patient No.	No. of days to CR	No. of days wbcs < 500/μl	No. of days platelets < 100,000/μl	Fever episodes*
		Age range, 20–39 yrs		
1	48	13	48	4
2	25	17	25	2
3	52	15	25	5
4	29	6	29	2
5	27	13	28	2
6†	—	—	—	1
7	28	14	17	1
8	28	15	25	2
9	46	2	45	1
10	55	9	32	3
		Age range, 40–59 yrs		
11‡	—	13	22	2
12‡	—	9	33	4
13	74	14	61	2
14	28	6	17	3
		Age, ≥ 60 yrs		
15‡	—	13	34	3
16‡	—	14	23	2
17‡	—	15	30	2
18	—	0	7	1
19	29	14	25	2
20	—	0	5	1
21	100§	23	49	1

*Sequential episodes of fever were separated by ≥ 24 hrs of temperature < 38°C.
†Died on Day 4.
‡Died while still pancytopenic.
§Second course given 8 wks after PR.

therapy. This regimen was most effective in the treatment of patients who were < 60 years old. Ninety percent of patients < 40 years old responded to therapy with an 80% CR rate. There was a 50% response rate in patients between 40 and 60 years of age. Of the four patients who were < 60 years old and who did not achieve CR status, three patients had AML which developed after therapy for a different primary neoplasm. Patients in the latter category frequently have leukemia which is refractory to cytotoxic chemotherapy.[5] If these patients are excluded from analysis, then the CR rate in patients < 60 years of age is 91% (100% for patients < 40 years and 67% for patients between 40 and 60 years). Patients > 60 years old did not fare as well. Five of seven patients died, two prior to completing a single course of chemotherapy.

Each of the eight patients who failed to respond to therapy died during induction therapy. Infection was the sole cause of death in four patients, two died from a combination of intracranial hemorrhage and infection, one died as a result of myocardial infarction, and one died from bleeding. Four of these patients died without evidence of leukemia on necropsy. Three of the four patients who died with persistent leukemia died after having received one complete course of chemotherapy.

The substitution of ADR for DNR provides an equally effective remission induction while exposing the patient to only two thirds of the total anthracycline dosage. It is generally agreed that anthracycline-induced cardiac damage is related to the total dose administered. Since on a mole-for-mole basis DNR and ADR seem to be equitoxic with respect to the heart (4), the use of the lower dose of ADR should permit the administration of a greater number of anthracycline treatments with safety than when DNR is employed in combinations with ara-C. A recent report from the Southwest Oncology Group employing ADR in combination with prednisone, vincristine, and ara-C has also demonstrated the efficacy of ADR in the treatment of AML (5). Hence, the early reports of the efficacy of ADR in the treatment of AML may have been too pessimistic (6).

[5]Preisler HD, and Lyman G. Acute myelogenous leukemia subsequent to therapy for a different neoplasm: chemical features and response to therapy. Submitted to *British Journal of Haematology*.

The high rate of remission induction in patients with de novo AML who are < 60 years of age is comparable to that achievable in childhood acute lymphocytic leukemia. It is hoped that remission-induction regimens such as the one reported here will render the treatment of de novo AML a problem of remission maintenance.

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Phase II Studies of Polychemotherapy Regimens in Small Cell Lung Cancer [1]

Brief Reports and Preliminary Communications

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Two recent reports (1,2) have demonstrated the efficacy of VP-16-213[3] in small cell undifferentiated lung cancer. Among the older, more standard agents, cyclophosphamide (CTX) and adriamycin (ADR)[4] are among the most active (3). *Cis*-dichlorodiammineplatinum(II)[5] (CDDP) is a new antineoplastic agent with primarily renal and auditory toxicity, and only minimal hematologic toxicity (4).

Based on these data, we elected to do phase II evaluations of three multiple-drug regimens: VP-16-213 plus CTX; VP-16-213 plus ADR; and CTX plus ADR and CDDP. The drug regimens were given as part of a combined chemoradiotherapy program in patients with previously untreated limited small cell lung cancer.

MATERIALS AND METHODS

Thirty-one previously untreated patients with small cell undifferentiated lung cancer were entered in the study. One patient voluntarily withdrew from the study prior to his first evaluation citing toxicity of the therapy as the reason. Of the 30 evaluable patients, 24 were men and six were women. The median age of this group was 65 years (range, 44–74 years). Using the Eastern Cooperative Oncology Group (ECOG) performance scale, seven patients were 0, 17 were 1, three were 2, two were 3, and one was 4.

All patients had clinical disease confined to one hemithorax and the ipsilateral supraclavicular fossas. Patients were staged on the basis of a physical examination, chest roentgenogram, and chemistry group. Bone roentgenograms and scans were used only if clinically indicated. Liver scans were obtained if the liver function tests were abnormal. If the scans were negative for tumor, no further evaluation was carried out. If the scans were either suspicious or diagnostic for tumor, a negative liver biopsy was required. All patients had normal computerized transaxial tomographic scans of the brain, both with and without contrast medium.

Two categories of disease were employed: "measurable" and "evaluable." Measurable disease required an indicator lesion with clearly measurable perpendicular diameters. Evaluable disease was visible tumor which could not be reproducibly measured because it lacked clearly defined perpendicular diameters. Using those two categories, 12 patients had measurable disease and 18 had evaluable disease.

Criteria for tumor response varied depending on whether measurable or evaluable disease was present. A complete regression (CR) was defined as a complete disappearance of all tumor and applied to both measurable and evaluable tumor. A partial regression (PR) was defined as a $\geq$ 50% reduction in the product of the perpendicular diameters of a measurable lesion. Regression (R) applied to evaluable disease and indicated a definite decrease in tumor agreed upon by at least two investigators. Stable disease (SD) was defined as a $<$ 25% increase or decrease in measurable disease agreed upon by two investigators. Progression of disease (PD) meant the appearance of any new lesion, a $\geq$ 25% increase in the size of a measurable lesion, or the definite increase in the size of an evaluable lesion agreed upon by two investigators.

Prior to randomization, patients were stratified by their ECOG performance score as 0–1 or 2–4. Patients with active cardiac disease were all treated with VP-16-213 plus CTX. Patients with renal disease (serum creatinine $>$ 1.2 mg/dl) were randomized between VP-16-213 plus CTX and VP-16-213 plus ADR. Patients with no cardiac or renal disease were randomized among VP-16-213 plus CTX, VP-16-213 plus ADR, or CTX, ADR, and CDDP.

Twelve patients were treated with VP-16-213 (70–75 mg/m^2 iv on Days 1, 3, and 5) plus CTX (600–675 mg/m^2 iv on Day 1); both drugs were repeated every 4–5 weeks. Seven of the 12 patients were entered randomly and five were entered directly because of

[1] Received Sept 30, 1976; revised Dec 13, 1976; accepted Dec 23, 1976.

[2] Mayo Clinic, Rochester, Minn.

[3] VP-16-213: NSC-141540; 4′-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside).

[4] Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8.

[5] *Cis*-dichlorodiammineplatinum(II): NSC-119875; CAS reg. No. 15663-27-1.

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cardiac disease. Nine patients had performance scores of 0–1 and nine were treated at the lower dose level.

Ten patients were treated with VP-16-213 as above plus ADR (40–45 mg/m² iv on Day 1); both drugs were repeated every 4–5 weeks. The last four patients entered were treated at the higher levels. Seven of the ten had performance scores of 0–1.

Eight patients were treated on Day 1 with CTX (400 mg/m² iv), ADR (40 mg/m²), and CDDP (30–40 mg/m² iv), and all drugs were repeated every 4 weeks. Six of the eight patients were treated at the higher CDDP dose. All eight patients had performance scores of 0–1.

The treatment plan was one cycle of chemotherapy followed by an evaluation in 4 weeks. Patients with tumors which regressed or were stable began radiation therapy at that time while continuing the same chemotherapy. Patients with PD were treated with either different chemotherapy alone or different chemotherapy plus radiation therapy, depending on the stage of their disease. All patients were scheduled for ten cycles of chemotherapy or a 450-mg/m² total cumulative dose of ADR, whichever came first. Following the completion of chemotherapy, patients were observed at 2–3-month intervals.

All pulmonary radiation therapy used either a 4- or 10-mv linear accelerator with a minimum skin-target distance of 100 cm. Anterior and posterior ports were used daily and included the primary tumor, the ipsilateral hilar nodes, and, if involved, the ipsilateral supraclavicular nodes. The mediastinal target area extended superiorly from the suprasternal notch to not < 5 cm below the carina inferiorly, and across the midline to include 1 cm of the contralateral lung; it was continuous with the primary target volume which included the involved portion of the lung with a maximum 3-cm margin. The midplane central axis dose in the thoracic region was 2000 rads in five fractions over 5–7 days, then a 3-week rest followed by a second 2000 rads in five fractions over 5–7 days. The maximal permissible dose to the spinal cord was 4000 rads in ten fractions over 5 weeks. Transverse and saggital isodose plots were obtained for each patient and anterior and posterior lead blocks were used to shield the spinal cord as indicated by the isodose plots.

RESULTS

Of the 30 patients evaluable after the first cycle of chemotherapy, 23 of 30 (77%) showed tumor regression. In these 23 patients there were one CR, 11 PRs, and 11 Rs. Six other patients were stable and only one progressed. With a mean followup time of 190 days (range, 81–378+ days), seven of 30 patients have died and no tumor was found in the only two patients autopsied (one patient died of CDDP-renal toxicity and one died of an acute myocardial infarction). The other five were felt to have PD. One other patient is alive with PD.

Of the 12 patients treated with VP-16-213 plus CTX, there were one CR, five PRs, three Rs, and three SDs after the first treatment for a regression rate of 75%. Following radiation therapy, the number of CRs increased to four (table 1). Three of the 12 patients are dead with tumor (81, 82, and 185 days). At present, the mean survival is 196+ days (range, 81–330+ days). Four patients are in the observation phase with a mean "no PD" interval of 85+ days (range, 10+–189+ days).

Among the ten patients treated with VP-16-213 plus ADR, there were two PRs, six Rs, one SD, and one PD after the first cycle of drugs for an overall regression rate of 80%. After radiation therapy, three patients achieved CR and one with SD achieved an R (table 1). The patient who progressed went to pulmonary radiation plus other polychemotherapy and is still alive at 289+ days. The mean

TABLE 1.—Response data following first cycle of chemotherapy and best response achieved during entire course of therapy

Drug	Regimen (mg/m²)	Response	
		After cycle No. 1	Best
VP-16-213 + CTX	70–75, Days 1,3,5 600–675, Day 1	1 CR, 5 PRs, 3 Rs, 3 SDs	4 CRs, 2 PRs, 5 Rs, 1 SD
VP-16-213 + ADR	70–75, Days 1,3,5 40–45, Day 1	2 PRs, 6 Rs, 1 SD, 1 PD*	3 CRs, 1 PR, 5 Rs
CTX + ADR + CDDP	400, Day 1 40, Day 1 30–40, Day 1	4 PRs, 2 Rs, 2 SDs	3 CRs, 2 PRs, 3 Rs

*Not included in best-response data.

survival for the ten patients is 202+ days (range, 71+–378+ days). Two of the ten patients are dead, one with central nervous system metastases and the other of an autopsy-proven acute myocardial infarction with no tumor found (241 and 244 days respectively). Only one patient has completed all ten cycles of chemotherapy and he remains disease free at 94+ days.

Following one cycle of CTX, ADR, and CDDP, six of eight patients (75%) showed tumor regression (four PRs and two Rs) and two had SD. Following irradiation, four patients were classified as CR (table 1). Two patients are dead, one with liver metastases and one from CDDP-renal toxicity with no tumor. The mean survival for the eight patients is 164+ days (range, 66+–280+ days). Only one patient has completed ten cycles of chemotherapy and he has not returned as yet for re-evaluation.

TOXICITY

Nausea and vomiting occurred on all three regimens; it was mildest in the VP-16-213 plus ADR regimen and was quite significant on the CTX, ADR, and CDDP regimen. One patient had a presumed myocardial infarction and shock following a severe episode of nausea and vomiting from VP-16-213 plus CTX. The CTX was discontinued and PD occurred at 173 days. One patient developed angina following the fifth cycle of VP-16-213 plus ADR. The ADR was discontinued, but the patient died of a myocardial infarction at 244 days. One patient died with renal failure at 180 days on the CTX, ADR, and CDDP schedule. This death was probably avoidable inasmuch as he was re-treated with CDDP in the face of an elevated creatinine of 1.9 mg/dl. One other patient had a mild elevation of the serum creatinine which reverted to normal following reduction in the CDDP dosage. There were no episodes of unequivocal ADR cardiotoxicity or ADR-augmented radiation dermatitis or esophagitis. Hematologic toxicity was mild in all three regimens with leukemia noted in 16 of 29 evaluable patients following the first drug cycle. With dosage adjustments, 24 of 30 patients developed wbc nadirs < 3000 cells/mm^3; platelet depression was infrequent and mild.

DISCUSSION

All three treatment regimens were quite effective and combined well with pulmonary irradiation. The major question which awaits an answer is what percentage of these patients may have been cured of their disease. Further observation of these patients following the completion of their ten cycles of chemotherapy is needed to answer that question.

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Adriamycin and Cyclophosphamide in Advanced Bladder Cancer [1,2,3]

Brief Reports and Preliminary Communications

Alan Yagoda,* Robin C. Watson, Harry Grabstald, Winston E. Barzell, and Willet F. Whitmore [4,5]

In clinical trials with adriamycin in advanced bladder cancer there were objective responses in 14%–27% (1,2). The addition of cyclophosphamide to adriamycin was synergistic in a carcinogen-induced (FANFT) bladder cancer model in mice (3). Therefore, a disease-oriented phase II clinical trial evaluated these two drugs in patients with advanced bladder cancer at Memorial Sloan-Kettering Cancer Center (MSKCC).

MATERIALS AND METHODS

Adriamycin and cyclophosphamide were given iv every 3 weeks to 17 patients with measurable metastatic bladder cancer and to three patients with urothelial (urethral, ureteral, and renal pelvic) tumors. Objective responses were assessed at 3-week intervals by physical examination and by evaluation of any abnormal results noted in liver function tests or a 12-channel screening profile. Blood counts were obtained routinely prior to each dose and during the first course, 7–14 days after therapy. Other studies included chest roentgenograms, bone surveys, iv pyelograms or loopograms, liver and bone scans, and lymphangiograms in patients with biopsy-proven lymph node involvement found at laparotomy. Roentgenograms were evaluated independently by one person, and pelvic and abdominal lesions were examined by two observers. An adequate trial was defined as two doses and a 1-month survival from the start of therapy or objective progression of disease after one dose. Partial remission (PR) denoted a $> 50\%$ reduction in the product of two diameters except for pelvic or abdominal masses which required a 75% reduction. Minor response (MR) included either a PR for < 1 month or 25%–50% tumor decrease persisting for > 1 month. Mixed responses or stabilization of disease for < 3 months were considered treatment failures. Duration was measured from the time response began. The Karnofsky performance scale (PS) was used to evaluate subjective changes.

There were 14 men and six women with a median age of 57 years (range, 39–75). The median PS was 75 and 45% of the patients were symptomatic. All patients had epidermoid (transitional cell) carcinoma except for one who had adenocarcinoma of urachal origin. Five patients had glandular or papillary features, and 47% had high-grade lesions. Eighty-five percent of the patients had had prior laparotomy and pelvic irradiation (2000–6000 rads), 60% had had an ileal conduit, and 45% had received irradiation to other areas. Six patients had had prior chemotherapy, all with *cis*-dichlorodiammineplatinum(II). The median time from diagnosis to metastases was 5 months and from metastases to protocol 8 months. Of the 20 patients accepted for the study, 14 (70%) had lung lesions and six (30%) had lymph node or skin lesions; two patients (10%) had abnormal lymphangiograms. Other involved sites in addition to the "indicator" lesions were evaluated (such as pelvic and rectal masses, 20%).

The initial doses were 45 or 60 mg/m^2 for adriamycin and 450–600 mg/m^2 for cyclophosphamide. Depending upon the degree of myelosuppression, subsequent doses were readjusted upward to (adriamycin/cyclophosphamide) 60/450, 60/600, 75/600, or 75/700 mg/m^2, or downward to 35/350 mg/m^2. Dosages and number of doses are outlined in table 1 showing 73 courses of adriamycin and cyclophosphamide administered to 20 patients. Three patients received one dose; three patients, two doses; six patients, three doses; four patients, four doses; and

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[3]Adriamycin: NSC-123127; CAS reg. No. 23214-92-8.

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

[4]Solid Tumor Service, Department of Medicine (Dr. Yagoda), Department of Diagnostic Radiology (Dr. Watson), and Urology Service (Drs. Grabstald, Barzell, and Whitmore), Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY.

[5]We gratefully acknowledge the cooperation of Dr. John Finkbeiner, the technical assistance of Miss Lucille Adinolfi, and the secretarial assistance of Mrs. Isa Irvin in the preparation of this manuscript.

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TABLE 1.—Adriamycin and cyclophosphamide dose and toxicity

Average dose in mg/m² of adriamycin/cyclophosphamide (range)	Total No. of patients	Total No. of doses	No. of evaluable doses for hematologic toxicity	Leukopenia*		Thrombocytopenia†	
				% of doses	Median nadir (× 10^3)	% of doses	Median nadir (× 10^3)
36/383 (40/450)	4	12	5	40	1.9 (0.5–3.3)	60	140 (127–149)
45/434 (40–50/375–500)	18	29	20	80	1.8 (0.9–3.6)	41	144 (100–172)
59/569 (55–65/450–600)	11	29	15	67	2.7 (1.4–3.9)	27	157 (135–182)
73/650 (70+/600+)	1	3	1	100	2.7	0	—

*< 4500 cells/mm³.
†< 175,000 platelets/mm³.

four patients, five to ten doses. The average total dose for adriamycin was 184 mg/m² (range, 45–430 mg/m²) and for cyclophosphamide, 1824 mg/m² (range, 450–4050 mg/m²).

RESULTS

Eighteen patients (90%) received an adequate trial and 17% achieved a PR. In the three responding patients, pulmonary, nodal, and skin lesions regressed for 5, 5, and 4 months, respectively. Two responders had had no prior chemotherapy and the third had achieved a PR lasting 5 months with *cis*-dichlorodiammineplatinum(II). Two of the three responders had had prior radiation therapy. When responders had progression, reinduction using higher schedules did not produce a second remission. All subjective and objective responses were evident within 3–4 weeks after the initial dose of adriamycin (45 mg/m²) and cyclophosphamide (450 mg/m²). No responses occurred with higher initial doses or subsequent increases in dosages.

Alopecia, vomiting, nausea, and anorexia, with resultant weight loss, were almost universal. There was no evidence of cardiac toxicity. Nine patients did not have cystectomy and two had no radiation to the bladder. Of the remaining seven patients, two (22%) experienced hematuria which, after cystoscopic examination, was attributed to cyclophosphamide toxicity. Since all patients had blood counts determined prior to each dose, but not routinely 7–14 days after subsequent doses (when the expected nadir should have occurred), the maximum extent of myelosuppression is not known. Life-threatening toxicity was noted in 44% of 18 adequately treated patients. Hospitalization was needed in 39% of the 41 evaluable courses due to severe myelosuppression and resulting sepsis.

DISCUSSION

The 17% response rate to the combination of cyclophosphamide and adriamycin administered iv every 3 weeks was disappointing. A previous study at MSKCC (1) using adriamycin singly noted responses in 14% of the patients. Two phase II drug-oriented trials (4,5) using this combination included four patients with urothelial tumors; no responses were obtained. In contrast, Merrin et al (6) found different results with cyclophosphamide used singly and combined with adriamycin in a disease-oriented clinical study in stage A–D (Jewett-Marshall) bladder cancer. Patients were "assigned" to either adriamycin (75 mg/m²), cyclophosphamide (1 g/m²), or adriamycin (40 mg/m²) and cyclophosphamide (200 mg/m² × 4 days orally); responses were noted in one of ten (10%), 11 of 21 (52%), and nine of 18 (50%) patients, respectively. They concluded that the addition of adriamycin to cyclophosphamide was not useful in obtaining more remissions than expected with cyclophosphamide alone.

The 52% remission rate with cyclophosphamide is high, but only four of 21 patients had stage D disease. Phase I, II (7,8), and disease-oriented trials (9,10) with iv cyclophosphamide in patients with advanced bladder cancer were reviewed and responses were evaluated using the same criteria employed in this paper. Only two PRs (7.4%) were noted in 27 patients (7–10). Twelve patients achieved an MR. At MSKCC one PR and one MR were obtained (unpublished data) in six patients with advanced bladder cancer.

From all available data, including the results of the present study, clinically useful (CR, PR) responses in urothelial tumors were obtained in up to 26% of patients (50 cases) treated with cyclophosphamide, 24% (> 175 cases) with adriamycin, and

30% (40 cases) with cyclophosphamide and adriamycin. These data suggest that the combination of cyclophosphamide and adriamycin probably yields response rates similar to those obtainable with each agent singularly and is distinctly inferior to *cis*-dichlorodiammineplatinum(II) alone (11) or in combination with cyclophosphamide.

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Use of DTIC in the Malignant Carcinoid Syndrome [1,2,3]

Anne Kessinger,* J. F. Foley, and H. M. Lemon [4]

Brief Reports and Preliminary Communications

Palliation of metastatic carcinoid tumors and the carcinoid syndrome with antineoplastic chemotherapy generally has been disappointing. A variety of drugs including cyclophosphamide, thioTEPA, mechlorethamine, 5-fluorouracil (5-FU), melphalan, actinomycin D, BCNU, mitomycin C, streptozotocin, hydroxyurea, DTIC, and adriamycin (1–7) have been used as single agents. The most favorable responses to single agents seem to occur with 5-FU (4), streptozotocin (6), and melphalan (5), and the more promising combinations include streptozotocin and 5-FU (4), and methotrexate and cyclophosphamide (8). Unfortunately, the responses are usually incomplete and short-lived.

Recently we had the opportunity to use DTIC in the treatment of two patients with advancing malignant carcinoid syndrome.

CASE REPORTS

Case No. 1

This patient was 57 years old in 1967 when she noted intermittent abdominal pain, which continued sporadically until November 1970 when it worsened, suggesting an intestinal obstruction confirmed at celiotomy on December 26, 1970. A 5-cm primary carcinoid tumor in the terminal ileum and eight adjacent lymph nodes containing metastatic tumor were resected. The liver appeared normal. In February 1971 intermittent facial flushing occurred. Liver scans revealed a metastatic lesion in the right posterior lobe. 5-HIAA excretion was 19 mg/24 hours (normal excretion = 2–15 mg/24 hours). She was referred to the University of Nebraska Medical Oncology Section. Therapy with oral cyclophosphamide and methotrexate and then with 5-FU was unsuccessful. By October 1971, liver scans showed a threefold increase in size of the metastases and 5-HIAA excretion rose to 50 mg/24 hours. Flushing persisted and diarrhea and intermittent palpitations appeared.

Streptozotocin (1.5 g/m^2 iv) was given once weekly for 4 consecutive weeks every 8 weeks. After the first course of streptozotocin, there was marked improvement in the diarrhea and flushing. In February 1972, liver scans showed a slight decrease in tumor size. The streptozotocin caused severe nausea and vomiting, and the patient preferred to stop the drug after the fourth course.

She did well until February 1973 when hepatosplenomegaly appeared. 5-HIAA excretion rose to 105 mg/24 hours and the liver scan suggested progression in tumor size and new lesions. DTIC (250 mg/m^2 iv) was given for 5 consecutive days every 28 days. After the first course, she experienced improved activity status and a decrease in flushing and diarrhea, and the liver scan showed appreciable improvement. DTIC was continued for 1 year. She became nearly asymptomatic, the liver scan continued to show improvement, and the 5-HIAA excretion dropped to 41 mg/24 hours in January 1974. Murmurs of pulmonary stenosis and tricuspid insufficiency appeared without symptoms. DTIC was stopped in February 1974 because she was doing well. In September 1974, DTIC was restarted because of evidence of progressing tumor and was stopped in February 1975 because of further tumor progression. Congestive heart failure appeared, requiring digitalization. She died in another hospital in September 1975 presumably of myocardial disease. An autopsy was not permitted.

Case No. 2

In 1972, a 55-year-old man developed diarrhea. In January 1974, flushing episodes associated with light-headedness appeared. In May 1974, he was noted to have a ruddy complexion, murmurs of aortic stenosis, mitral insufficiency, an abnormal liver scan, and 5-HIAA excretion of 120 mg/24 hours. At celiotomy a 10-cm mass foreshortening the ileal mesentery and multiple hepatic nodules up to 10 cm

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[2]DTIC: NSC-45388; CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-.

[3]Supported in part by Public Health Service grant CA-19623 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

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in diameter were biopsied, confirming the diagnosis of metastatic carcinoid tumor.

He was referred to the University of Nebraska Medical Oncology Section and streptozotocin (1.5 g/m² iv weekly for 4 consecutive weeks every 8 weeks) was begun. After one course, the 5-HIAA excretion fell to 17.2 mg/24 hours. After two courses, flushing was less frequent but diarrhea continued. After four courses, in October 1974, the liver scan revealed a 50% increase in tumor size but the 5-HIAA excretion was only 16.6 mg/24 hours. Because of the dramatic change in liver scan, not accompanied by chemical or clinical changes, therapy was changed to DTIC (250 mg/m² iv for 5 consecutive days every 28 days) and continued for 1 year. Serial liver scans showed no further disease progression. 5-HIAA excretion varied and did not always correlate with his clinical course of progressive weight gain, disappearance of flushing, and decrease in stool frequency to one or two a day.

In October 1975 DTIC was reduced to 250 mg/m² iv on 5 consecutive days every 42 days. A liver scan in July 1976 showed a slight decrease in the size of the liver metastases. The patient is presently working full-time on his farm.

DISCUSSION

DTIC is not a drug generally used in the treatment of metastatic carcinoid, although Davis et al (4) have alluded to one such patient treated with DTIC who did not respond. DTIC was administered to the first patient reported here after nearly all drugs reported to be helpful had been used and her tumor continued to grow. The drug was selected because it is somewhat effective in the treatment of malignant melanoma, a tumor whose cellular embryologic origin is the same as that of the carcinoid tumor, the neural crest. DTIC was selected for the second patient after streptozotocin failed because of the good response demonstrated by the first patient.

Both of these patients experienced a slight, short-lived worsening of flushing and diarrhea following DTIC administration. A temporary worsening of the syndrome has been reported by others during and just after effective antineoplastic chemotherapy is given, and is thought to be produced by escape of tumor products into the circulation as the malignant cells are destroyed (5). This sudden flare can be fatal in patients with significant heart disease (4).

The first patient showed a definite decrease in 5-HIAA excretion during her remission with DTIC, but the second patient did not demonstrate a drop in 5-HIAA excretion following arrest of tumor growth. Others have noted that some patients may have remission using various drugs without a concomitant change in 5-HIAA excretion (6,8). Thus, 5-HIAA excretion is not an accurate means of following carcinoid tumor status.

Non-antineoplastic pharmacologic therapy for symptomatic palliation of the syndrome is sometimes helpful, but rarely if ever produces complete regression of symptoms. The best palliation of the syndrome would seem to depend on actual decrease or disappearance of tumor. The response of the first patient was disappointing because, in spite of objective evidence of decreased tumor size and reduction of 5-HIAA excretion, she died of progressive carcinoid heart disease. This suggests that long-term palliation may not be possible unless the tumor becomes completely nonfunctioning.

DTIC is another antineoplastic agent that may be useful in the management of metastatic carcinoid tumors, and combinations of this drug with other drugs of proven value seem warranted.

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LETTERS

Toxicity of Cancer Chemotherapeutic Agents in Athymic (Nude) Mice

Several animal models have been developed and used to transplant and grow human tumors for assessment of the antitumor effect of cancer chemotherapeutic agents. These include the hamster cheek pouch (1), thymectomized mice (2), and the athymic (nude) mouse (3,4). The nude mouse model would appear to be ideal for these studies because a variety of human tumors are accepted and can be serially transplanted, and no preparation of the animals is necessary for transplantation.

An increasing number of laboratories are employing the nude mouse for growth and treatment of human tumors. Little toxicity data for standard chemotherapeutic agents in nude mice have been reported (4). Therefore, a number of these agents were tested over a dosage range to determine toxic levels.

Male nude mice of a National Institutes of Health (NIH) Swiss background were obtained from the Division of Cancer Treatment (DCT), NCI colony at the Frederick Cancer Research Center, Md. They were maintained under filter-top cages with autoclaved food and bedding and acidified water (pH 2.0). Male NIH Swiss mice were obtained from the colony at NIH. All of the mice were 6 weeks old (20–25 g) at the initiation of the experiments.

The drugs were supplied by the Developmental Therapeutics Program, DCT, NCI. They were dissolved to give the desired concentration and injected by the ip route in a volume of 0.01 ml/g of body weight of the mice. Seven mice were employed for each dose level. The animals were observed for mortality for 30 days. Conventional mice of the same age and weight range were used as controls for each drug.

The toxicity of a series of antitumor drugs in nude and conventional Swiss mice is compared in table 1. The LD50 doses, as determined by Karber's method (5), were significantly higher in nude than in Swiss mice for BCNU, methotrexate, and 5-fluorouracil (5-FU). The toxic doses were somewhat higher in nude mice but not significantly so for bleomycin and vincristine. The toxic doses were the same in both strains for adriamycin, cyclophosphamide, actinomycin D, and vinblastine while DTIC was the only drug which was somewhat more toxic in nude than in conventional mice.

It was not expected that nude mice would be able to tolerate at least as much or higher doses than conventional mice of some cancer chemotherapeutic agents. This points out the need of assessing the toxic levels of agents in the particular strain of nude mice that are to be employed for tumor studies. Since the tolerated doses may be higher, as seen in these experiments, it may be possible to use higher doses for treatment of the tumor under investigation.

No data are available to explain the ability of the nude mice to tolerate higher doses of some drugs. Since the toxicity of some of these agents is due to immunosuppression or myelosuppression, the nude mice may have some advantage by the

TABLE 1.—Toxicity of antineoplastic agents to male NIH Swiss and nude mice

Drug	Schedule	LD50 (95% confidence interval) (mg/kg/injection, ip)	
		Conventional Swiss mice	Nude mice
BCNU	Single dose	32.3 (25.1–41.6)	66.9 (48.3–92.9)
Methotrexate	Qd × 5	1.94 (1.32–2.80)	3.24 (2.86–3.75)
5-FU	Q4d × 3	51.9 (40.9–65.8)	80.3 (66.6–97.0)
Bleomycin	Single dose	53.8 (34.7–83.4)	96.4 (72.0–129)
Vincristine	Single dose	3.23 (2.44–4.27)	4.32 (3.73–5.00)
Adriamycin	Single dose	14.4 (12.4–16.6)	14.4 (11.2–18.5)
Cyclophosphamide	Single dose	159 (108–236)	159 (108–236)
Actinomycin D	Single dose	0.80 (0.67–0.97)	0.75 (0.61–0.92)
Vinblastine	Single dose	3.61 (2.50–5.22)	3.12 (2.43–4.02)
DTIC	Qd × 5	232 (210–254)	187 (145–240)

fact that they may have more developed alternate immune pathways. Nude mice have been shown to have both a highly developed natural cytotoxic response to tumor cells by in vitro tests employing spleen cells as effectors (6) and a well-developed anti-Hh allograft response (7). Recently, Cutler (8) reported that nude mice were more resistant than normal littermates to a lethal dose of *Candida albicans*. Another possibility may be that the nude mice have some differences in metabolism compared to conventional mice. Experiments by Litterst (personal communication) have shown that there is more activity of the hepatic microsomal drug metabolizing enzyme system in nude mice than in conventional Swiss mice. In similar experiments Freudenthal et al (9) also observed this and in addition they found that the activity of the microsomal enzyme system is the same for nude and Swiss mice that are heterozygous for the nude gene.

LIST OF COMPOUNDS

Actinomycin D: NSC-3053
Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-
Bleomycin: NSC-125066; CAS reg. No. 11056-06-7; [2,4'-bithiazole]-4-carboxylic acid, 2'-(2-aminoethyl)-, monohydrate
Cyclocytidine: NSC-145668; CAS reg. No. 10212-25-6; 6*H*-furo[2',3':4,5]oxazolo-[3,2-*a*]pyrimidine-2-methanol, 2,3,3a,9a-tetrahydro-3-hydroxy-6-imino-, monohydrochloride
DTIC: NSC-45388; CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-
5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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November 8, 1976

Contributions to *Cancer Treatment Reports* From Private Practitioners

I am writing to propose that you develop a special section within *Cancer Treatment Reports* devoted to the publication of papers written by private practitioners or with particular relevance to the practicing medical oncologist. At present, no cancer journal has addressed itself in a real way to this segment of the oncologist population, and I think it would be especially appropriate to have an NCI-sponsored journal lead the way.

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December 30, 1976

Editor's Reply:

The editorial board would welcome articles relevant to the special problems of the practice of medical oncology. This material would be reviewed by persons considered expert in the subject matter, and would be measured by the same criteria of excellence and relevance used to judge other manuscripts. At present the number of manuscripts does not justify creating a special section to deal with this area of interest.

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A Possible Neurologic Complication of DTIC[1]

DTIC is currently the most successful chemotherapeutic agent in the treatment of disseminated malignant melanoma. It is being widely used either as a single agent or in combination with other drugs (1,2). Gams and Carpenter (3) have described central nervous system complications arising in three patients receiving DTIC chemotherapy. These patients suffered from epileptic seizures; in one patient no evidence of cerebral metastatic disease was found after extensive investigation, although in two other patients cerebral metastases developed later. The authors suggested that these neurologic complications might be related to combination treatment with adriamycin and DTIC. Earlier, Gerner et al (4) described cerebral hemorrhage, not related to thrombocytopenia, in patients receiving a combination of DTIC and actinomycin D. These patients, who had no previous evidence of cerebrovascular disease and no apparent cerebral metastases, had a syndrome consisting of acute onset of headache with hemiparesis, with rapid deterioration until death occurred.

We would like to describe the case history of a patient with stage 3B (M. D. Anderson staging classification) malignant melanoma who was treated by surgery to the involved regional nodes followed by intermittent chemotherapy with DTIC and BCG as immunotherapy. The patient developed a severe dementia necessitating institutional care and recently died with no evidence of malignant melanoma.

Case History

In 1972, a 46-year-old livestock specialist had an enlarging mole on his abdomen removed. This was shown to be a malignant melanoma. He had diabetes mellitus which had been well controlled by insulin for 26 years. He remained well until February 1974, when a tumor developed in his right axilla which was excised and found to be metastatic malignant melanoma. In June 1974, he complained of left-sided facial weakness and was found to have, in addition, slurred speech and some weakness of the left upper and lower extremities. A brain scan was negative, but an electroencephalogram at that time showed a mild dysrhythmia most evident in the right frontal region. There was no evidence of diabetic complications and a cerebral metastasis of malignant melanoma was considered a strong possibility. However, by August 1975, he made a complete recovery with no neurologic abnormality detectable at that time from a clinical examination. He received two cycles of chemotherapy with DTIC at a dose of 500 mg × 5 days each month plus intradermal and oral BCG, and one cycle with DTIC at a dose of 700 mg × 5 days as treatment for his proven stage 3B malignant melanoma. This chemotherapy caused severe nausea lasting 1–2 weeks after the end of a course of treatment.

After 3 months of chemotherapy it was noted that the patient was irascible, depressed, and mildly confused, particularly when he was receiving treatment with DTIC. After one more course he refused further chemotherapy and was thereafter followed on a routine basis.

In April 1975, he was seen again because of deterioration of his memory. His wife observed deterioration, particularly from the time of chemotherapy, although she felt he had displayed a degree of absentmindedness for up to a year beforehand. The memory impairment was confirmed after examination and the minimal left hemiparesis was noted to be present again. Extensive investigation showed no evidence of recurrent malignant melanoma. The brain scan, skull roentgenogram, and lumbar puncture were negative. Computerized axial tomography showed a minimally dilated ventricular system. A computerized axial tomogram with augmentation was performed and this suggested the presence of frontal and right parietal cortical atrophy. An electroencephalogram was abnormal with generalized dysrhythmias and a suggestion of a focal lesion in the frontal regions.

This patient's intellectual function continued to deteriorate despite a trial of vitamin B therapy and good control of his diabetes, and eventually he required institutional care.

In November 1975, he died and a full autopsy including examination of the brain was carried out. The cause of death was myocardial infarction. There was no evidence of recurrent malignant melanoma, either in the brain or elsewhere. Examination of the brain showed mild dilatation of the ventricles with some frontal cortical atrophy. There was no evidence of cerebrovascular disease. Nonspecific, sub-arachnoid fibrosis was seen on microscopy.

DTIC has the structural characteristics of a purine antagonist and the biologic characteristics of an alkylating agent (5). After iv injection it is detectable in the cerebrospinal fluid at levels of 14% of the serum concentration, although it is

[1] DTIC: NSC-45388; CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-.

relatively lipid insoluble (6). It is metabolized by the liver to 5-amino-4-imidazole carboxamide.

Bone marrow suppression is generally predictable, although there have been some reports of idiosyncratic hematosuppression which is particularly severe in certain patients. Nausea and vomiting are often associated only with the initial injections of a course. Facial flushing and paresthesias have been described (7) in addition to the suggested neurologic complications described earlier.

The patient whose case history is described above developed a severe dementia after receiving DTIC to which he had reacted badly with severe nausea and vomiting, confusion, and depression. While it is possible that the dementia was due to an unrelated organic pre-senile dementia, the evolution of the illness was not typical of this and appeared to have been exacerbated by DTIC. In view of this and other reported cases of central nervous system complications associated with DTIC administration, we would suggest extreme caution in the use of DTIC in patients with pre-existing cerebral disease or in patients who exhibit neurologic abnormalities while taking the drug. We hope this case is an isolated one, but we are anxious to hear from any other investigators who may have observed similar cases of delayed dementia.

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Response of Medullary Thyroid Carcinomas and Carcinoid Tumors to Adriamycin[1]

Solomon et al (1) recently reported the rapid response of a metastatic malignant carcinoid tumor to adriamycin. This response is not surprising in view of the very favorable experience previously achieved by Gottlieb and Hill (2,3) in patients with medullary thyroid carcinoma treated with adriamycin. Medullary thyroid carcinoma and carcinoid tumors are known to be embryologically related (4) and to share common histologic, ultrastructural, cytochemical, and humoral properties (5).

Gottlieb and Hill (3) treated 43 patients who had various histologic types of metastatic thyroid carcinoma with adriamycin. They observed that remissions occurred most frequently and were longest in patients with medullary carcinomas. Of six patients with medullary carcinomas, three exhibited objective regressions with a median duration of response of > 21 months and a median survival of > 26 months. Of the remaining three patients, two who had associated diarrhea showed no significant change in their measurable disease, but experienced complete relief of the diarrhea. The sixth patient survived only 3 days.

Adriamycin, alone or in combination with other drugs, should receive a more extensive evaluation in the treatment of metastatic malignant carcinoid tumors, medullary thyroid carcinoma, islet cell carcinoma, and other "apudomas."

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[1]Adriamycin: NSC-123127; CAS reg. No. 23214-92-8.

similarities of carcinoid tumors and medullary carcinomas of the thyroid. Surgery 74:21–29, 1973.

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The Carcinoid Syndrome—A Role for Hemodialysis? [1]

The carcinoid syndrome includes varying degrees of cutaneous flushing, intestinal hypermotility, bronchoconstriction, and valvular heart disease. It is generally agreed that the syndrome results from the secretion by the tumor of 5-hydroxyindoles which include 5-hydroxytryptamine (serotonin), 5-hydroxytryptophan (5-HTP), and perhaps other agents (1). An unusual complication of chemotherapy has been a worsening of these symptoms, somnolence, and even death, possibly from the sudden release of serotonin or related compounds (2). Therefore, in patients with severe clinical manifestations or 5-hydroxyindoleacetic acid (5-HIAA) excretion > 150 mg/24 hours (normal = < 10 mg/24 hours), we have reduced the initial dose of chemotherapy by 50%. The following observations suggest that hemodialysis may be of value in the management of the life-threatening carcinoid crisis.

A 48-year-old man with biopsy-proven carcinoid tumor in the liver had a 2-year history of a 50-pound weight loss, 2–7 diarrheal stools/day, and facial flushing. Pertinent physical findings included facial telangiectasias and a liver which measured 9 cm below the right costal margin in the midclavicular line and 10 cm below the xiphoid. The urinary 5-HIAA excretion was 507 mg/24 hours and the whole blood 5-hydroxyindoles were 1.85 μg/ml (normal = <0.3 μg/ml) (3). Since the patient was at high risk for a carcinoid crisis, the feasibility of using hemodialysis was tested by dialyzing a sample of the patient's whole blood and plasma with a dialysate containing the following (measured in mEq/liter): Na, 140; Cl, 110; Ca, 3; K, 2; glucose, 110; and acetate, 35. 5-Hydroxyindole concentrations were reduced in whole blood and decreased to undetectable levels in plasma after dialysis (table 1).

TABLE 1.—Total hydroxyindoles

Before dialysis	After dialysis
1.85 μg/ml whole blood	0.38 μg/ml
1.56 μg/ml plasma	0

Previously it was found that there was little difference in pre- and post-dialysis levels of serotonin in blood in a study of 23 long-term dialysis patients with renal failure (4),[2] probably a result of serotonin being retained by platelets and perhaps being bound to plasma protein. However, a large proportion of the 5-hydroxyindoles were present in the plasma of our patient (table 1). This suggested that his tumor was not secreting serotonin but was secreting its precursor, 5-HTP, which has not been shown to be concentrated by platelets. Analysis of another sample of his blood (3) showed that large amounts of 5-HTP (1.34 μmg/ml) were indeed present. We also found that when 5-HTP (10 μmg) was added to an aliquot (4 ml) of our patient's blood it was entirely removed by the hemodialysis procedure. Since 5-HTP crosses the blood-brain barrier with ease and causes an accentuated drowsy electroencephalographic pattern in laboratory animals (5), it may be a factor in the somnolence which has been noted by one of us (6) and by others in some carcinoid patients undergoing chemotherapy.[3] Although pharmacologic maneuvers of α-adrenergic blockade, corticosteroids, and serotonin antagonists may be of some value in treatment (7), hemodialysis would seem to offer another therapeutic alternative in the management of this challenging problem, particularly in those patients with tumors that secrete 5-HTP.

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[1]Supported in part by grant NS-9143 [GMT].

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Metastatic Mediastinal Carcinoid Treated With Chemotherapy

Carcinoid tumors arising in the mediastinum have recently been reported (1) and a very aggressive clinical course has been described in some patients in whom the mediastinal carcinoid tumor has been associated with the multiple endocrine adenomatosis syndrome (2). We recently saw a 25-year-old man who presented with a widely metastatic carcinoid tumor apparently from a mediastinal primary, and in whom no evidence for the multiple endocrine adenomatosis syndrome could be found, either initially or at post-mortem examination. Nevertheless, his tumor pursued an extremely aggressive course and despite remissions following two different chemotherapeutic regimens, he died 9 months after presentation.

Although considerable attention has been directed toward pharmacologic control of the carcinoid syndrome, there has been only limited experience with antitumor chemotherapy of the carcinoid malignancy. The patient reported here had a pronounced decrease in the size of his mediastinal mass and pulmonary infiltrate following therapy with cyclophosphamide (20–30 mg/kg iv every 2 weeks) and vincristine (2 mg weekly).[1] After a 3-month remission, however, he developed progression of the mediastinal and pulmonary masses and rapid growth of the cervical and supraclavicular nodes. He received 3600 rads of radiation therapy to the mediastinum and right lung and 2400 rads to right supraclavicular and cervical lymph nodes, and the tumor masses regressed partially. Adriamycin (60 mg/m^2 iv every 3 weeks) resulted in a further decrease in the size of the lymph nodes and chest masses.[2]

After a 3-month remission on adriamycin he developed widespread osteoblastic metastases, generalized bone pain, increased adenopathy, and retinal hemorrhages. Despite a trial of BCNU chemotherapy his condition deteriorated rapidly and he died.

Post-mortem examination revealed carcinoid tumor involving the mediastinum, lungs, lymph nodes, liver, adrenals, pituitary, bone, and right optic nerve. Careful examination to rule out a carcinoid tumor of bronchial, pancreatic, or gastrointestinal origin was negative.

This patient had a dramatic subjective and objective response to a combination of cyclophosphamide and vincristine and subsequently to radiotherapy followed by adriamycin. We suggest that a combination of these agents should be tried in similar cases of aggressive carcinoid tumors.

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January 24, 1977

[1]Cyclophosphamide: NSC-26271; CAS reg. No. 6055–19–2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Vincristine: NSC-67574; CAS reg. No. 2068–78–2; leurocristine, sulfate (1:1).

[2]Adriamycin: NSC-123127; CAS reg. No. 23214–92–8.

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ANNOUNCEMENTS

Second Conference on Lung Cancer Treatment

Airlie House, Virginia, May 22–24, 1977

This conference is sponsored by the Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, Md). Current trends in the treatment of lung cancer will be analyzed and prospects for further therapeutic approaches will be discussed. Workshops are planned for the following topics: Pathology (chairmen: M. Matthews and R. Yesner), Surgery (C. Mountain), Adjuvant Chemotherapy (G. Bonadonna), Adjuvant Immunotherapy (R. Ritts), Staging and Followup (H. Hansen), Radiotherapy (C. Perez), Chemotherapy of Non-Small Cell Carcinoma (P. Alberto and M. Cohen), Small Cell Carcinoma (Y. Kenis and J. Minna), Tumor Immunology (P. Chretien), Experimental Chemotherapy and Kinetics (F. Schabel), and New Modalities (P. Rubin). Each workshop will be introduced with invited papers. Some papers will also be selected from submitted abstracts. Attendance will be limited to 120 persons. There is no registration fee.

For further information contact: Dr. Franco M. Muggia, Second Conference on Lung Cancer Treatment, National Cancer Institute, Bldg 37, Rm 6A17, National Institutes of Health, Bethesda, Md 20014.

Symposium on "Use of Athymic (Nude) Mice in Cancer Research"

Columbus, Ohio, June 15–16, 1977

The Battelle Memorial Institute (Columbus, Ohio) and the Division of Cancer Treatment of the National Cancer Institute (Bethesda, Md) announce a symposium on "Use of Athymic (Nude) Mice in Cancer Research" (Drs. David Houchens and Artemio Ovejera, co-chairmen) to be held at Battelle Memorial Institute, 505 King Ave, Columbus, Ohio, on June 15–16, 1977.

This symposium will cover topics of mouse development, biology of nude mice, immunologic studies, tumor development, and treatment of tumors.

For further information and registration forms write to: Ms. Susan Armstrong, BATTELLE, Columbus Laboratories, 505 King Ave, Columbus, Ohio 43201.

American Board of Internal Medicine Certifying Examination in the Subspecialty of Medical Oncology

October 18, 1977

The American Board of Internal Medicine will administer the Certifying Examination in the Subspecialty of Medical Oncology on Tuesday, October 18, 1977. Application forms may be requested beginning January 1, 1977.

Requests for information and application forms should be directed in writing to: Registration Department, American Board of Internal Medicine, 3930 Chestnut St, Philadelphia, Pa 19104.

Postgraduate Course on Gynecologic Tumors

The National Cancer Institute of Milan and the Italian League against Cancer, Milan Section, will organize a Postgraduate Course on Gynecologic Tumors to be held June 13–17, 1977 (*Chairmen:* Prof. U. Veronesi and Prof. A. Perussia; *Coordinator:* Prof. F. Di Re; *Secretary:* Dr. M. De Lena).

The subjects of the lectures are the epidemiology, etiology, pathology, diagnostics, clinical classification, and treatment (surgical, radiologic, medical, combined, antalgic) of gynecologic tumors. A large group of Italian experts will give their co-operation to the following foreign lecturers: Chassagne, Muñoz (France); Stoll (Germany); Tj Kuipers (Netherlands); Davy

(Norway); Walstam and Zajicek (Sweden); Bagshave and Joslin (UK); and Fletcher, Woodruff, and Young (USA). Three panel discussions will be held entitled "Cytological Organization of Mass Screening"; "Diagnosis of Ovarian Neoplasias"; and "Ovarian Cancer: What Type of Therapy?". There will also be closed circuit television presentation of surgical operations.

Simultaneous translations will be provided. The subscription fee is Lit. 100.000 (covering the course, proceedings, lunch, etc). *As the number of participants is limited, please send your subscription as soon as possible to:* Miss Lucia Manfredi, Ufficio Attività Didattiche, Istituto Nazionale per lo Studio e la Cura dei Tumori, Via G. Venezian, 1, 20133 Milano, Italy (tel. 2363764).

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